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SOME EFFECTS OF SYNTHETICALLY PREPARED MALE HORMONE (ANDROSTERONE) IN THE RAT[1, 2]

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An outline of progress on the male hormone problem might be as follows: *a*) the demonstration by Berthold (1), in 1849, of an internal secretory action by the testis; *b*) emphasis directed towards hormone secretion and its function between 1900-1920 (2); *c*) the first successful extraction of the hormone from testicular tissue in 1927 by McGee (3); *d*) the crystallization of a male hormone, androsterone, from human urine by Butenandt in 1931 (4); *e*) the preparation in 1934 of androsterone by synthetic means from cholesterol by Ruzicka (5); *f*) the preparation in 1935 of a crystalline male hormone, testosterone, from fresh testicular tissue by David, Dingemanse, Freud and Laqueur (6); *g*) the preparation by synthetic means of testosterone in 1935 by Butenandt and Hanish (7) and Ruzicka and Wettstein (8) as well as the preparation of many other substances possessing androgenic activity (for review see 9).

In addition, many facts have accumulated with regard to proper indicators for male hormone, perfection of methods of extraction, standardization, concentration, purification, chemical properties and the relation of active compounds to other similar organic compounds, as well as the biological effects of these various substances within the organism.

The availability of androgenic substances in pure crystalline compounds, such as androsterone, dehydroandrosterone, testosterone and others, makes important a study of their effects in comparison with the effects of the natural hormone secreted by the testis. Through the courtesy of Professor L. Ruzicka, C. C. Haskell and the Ciba Company there was made available to us 1.8 grams of crystalline androsterone prepared by synthetic means and this report concerns itself with observations on the effects obtained from injections of

[1] This investigation has been aided by a grant from the Rockefeller Foundation to the University of Chicago.
[2] Read before the Twentieth Annual Meeting of The Association for the Study of Internal Secretions, Kansas City, Kansas, May 12, 1936.

this preparation into rats. It is a pleasure to acknowledge our gratitude for the opportunity of studying these effects at first hand.

RESULTS

It has been the aim of this study to determine some of the effects of androsterone when injected subcutaneously in oil solution into castrate and normal male rats from prepuberal ages to mature adults, and to compare the results with those obtained after injections of bull testis extracts reported earlier.

This report is based upon careful study of 15 litters of rats consisting of 87 males of known ages, and in addition 21 adult males and 10 females of similar age but not known to be littermates. The 108 males comprised 36 normal and 17 castrate controls, 33 castrates and 22 normal males receiving androsterone treatment. In all cases excepting the adult group the results were determined by a comparison of injected males with littermate controls (normal and castrate). Results obtained from treatments of 8 male ground-squirrels will be reported elsewhere (10).

A. Treatments immediately after castration

It may be recalled from earlier work (11), (12), that the prostate gland and seminal vesicles of sexually mature rats show cytological involution changes within 2 to 5 days after castration, that castration changes progress rapidly to a typical low level within approximately 2 weeks, and that bull testis extract prevented the appearance of involution changes. Administration of larger than maintenance doses produced organs of larger size than in the normal controls. Thus there was in these respects complete substitution for the hormonal activity of the testis by injected materials.

Crystalline androsterone was administered by daily injections for the usual 20-day periods beginning the day following testis removal. In table 1 results are given on litters receiving treatments at ages from 15 to 35 days, 35 to 55 days and mature adults. At 35 days of age, normal males have just begun to show secretory differentiation in the seminal vesicle epithelium whereas the secretory state of prostate epithelium has been developed before the 20th day. At 55 days of age both glands are actively secreting and contain the characteristic epithelium of a normal adult organ. Whereas the 3 lobes of the prostrate gland were each weighed and studied, notations here are restricted to the ventral lobes unless otherwise stated.

Age 15 to 35 Days. The seminal vesicles of castrate males treated daily with 1 mg. of androsterone were found to be much more highly developed in secretory activity than those of normal littermate controls. The secretory epithelium was assuming the character typical for a much older animal and secretion granules were present; the normal control had not yet reached a well developed secretory state. Injections of 2 mg. and 3 mg. daily produced more pronounced secretory differentiation in seminal vesicles. In fact, after 3 mg. injections these were indistinguishable from similar tissue of adult normal males, although secretory granule differentiation was stronger than that found

TABLE 1. ANDROSTERONE TREATMENTS IMMEDIATELY FOLLOWING CASTRATION.

	Group No.	Treatment	Wt. ventral (post.) prostate, gm.	Wt. (2) sem. ves. gm.	Histological Rating[1] prost.	Histological Rating[1] sem. ves.	Ratio, prostate/ sem. ves.
		Castrated, day 15. Daily injections, day 15–34. Sacrifice, day 35.					
1.	143	cast. cont.	.0330	.0034			17
	144	cast. cont.	.0320	.0030			19
	140	1.0 mg.	.1276	.0192			12
	141	2.0 mg.	.1470	.0668			5
	142	3.0 mg.	.1220	.2520			1
	135	norm. cont.	.0402	.0050			17
	136	norm. cont.	.0436	.0056			17
	137	norm. cont.	.0470	.0072			12
		Castrated, day 35. Daily injections, day 35–54. Sacrifice, day 55.					
2.	72	cast. cont.	.0118	.0066	0	0	
	73	0.1 mg.	.0584	.0132	***	0	
	74	0.3 mg.	.0376	.0122	***	0	
	75	0.5 mg.	.0990	.0238	****	***	
	76	norm. cont.	.0870	.0944	****	****	
		Castrated, day 35. Daily injections, day 35–52. Sacrifice day 53.					
3.	154	1.0 mg.	.1640	.0166	****	***	20
	155	2.0 mg.	.1490	.0278	****	***	14
	156	3.0 mg.	.2346	.1306	****	****	4
	150	norm. cont.	.0920	.0422	****	****	5
	152	norm., ¼ cc. oil	.0848	.0358	****	****	6
		Adults; castrated 20 days; 19 daily injections; body wt. 220–300 gm.					
4.	68	cast. cont.		.2040	0	0	
	69	cast. cont.		.1920	0	0	
	62	0.1 mg.		.1750	**	*	
	63	0.1 mg.		.1806	**	0	
	64	0.3 mg.		.1862	***	0	
	65	0.3 mg.		.1964	***	**	
	66	0.5 mg.		.2474	****	***	
	67	0.5 mg.		.2468	****	***	
	70	norm. cont.		1.0188	****	****	
	71	norm. cont.		1.0836	****	****	
		Adults; castrated 20 days; 19 daily injections; body wt. 190–270 gm.					
5.	43	0.5 cc. oil		.1170	0	0	
	44	0.5 cc. oil		.1456	0	0	
	37	0.5 mg.		.2164	****	***	
	38	0.5 mg.		.1980	****	***	
	39	1.0 mg.		.2920	****	***	
	40	1.0 mg.		.3210	****	****	
	41	2.0 mg.		.7782	****	****	
	42	2.0 mg.		.2684	****	****	
	45	norm. cont.		1.2660	****	****	
	46	norm. cont.		1.0890	****	****	

[1] Histological ratings grade from 0 (no effect from treatment) to ****, the normal condition. Group 1 not rated since tissues from all treated males were more developed than the controls. Prostate/seminal-vesicle ratio determined by dividing wt. of prostate complex (not given in table, see graph) by wt. of seminal vesicles.

in most untreated normal adult males. Figures 1, 2 and 3 display the character of seminal vesicle epithelium in the castrate and normal control and in the castrate receiving daily injections of 3 mg. androsterone.

Comparisons of fresh weights of the seminal vesicles reveal those of treated

castrates to be greater than those of the normal controls—3 times as great after 1 mg. treatments and 50 times as great after 3 mg. treatments.

The prostate gland, as represented by the ventral lobe, cannot be properly evaluated at 35 days of age except by gross weight responses. Price (13) has shown that castrations within a few days after birth, in contrast to castrations of sexually mature males, fail to inhibit the differentiation of the prostate secretory epithelium until an attained age of 35 to 40 days. The contention is confirmed in these experiments since the prostate glands of these 35-day-old castrates possess the well developed light areas diagnostic for active secretory cells of this lobe; the character of the secretory epithelium was but little changed by androsterone injections and all prostates of this litter were rated normal in histological character.

Fresh weights of the prostate glands of the series show that those of untreated castrates were but slightly below those of untreated normals. Injected castrates, however, showed increased prostate size, since with the 3 dosages employed there was an increase to 3 times or more the weights of this lobe from normal untreated littermate controls.

Age 35 to 55 Days. Androsterone was administered daily to animals castrated for this period in dosages ranging from 0.1 mg. to 3.0 mg. (see groups 2 and 3, table 1). Both the prostate gland and seminal vesicles of normal males at 55 days showed an active secretory condition.

Seminal vesicle epithelium was maintained in an approximate state of normal secretory activity in this period with daily treatments of 0.5 to 1 mg. of androsterone. Secretion granules were present after such dosages but were usually somewhat weaker than those from normal littermate controls. Larger dosages resulted in stronger development of the secretory activity. In fresh weight comparisons for this age period, 2 mg. daily produced somewhat smaller organs in the treated castrates than those of normal littermate controls whereas 3 mg. daily produced organs in the castrates 3 to 4 times the weight of normal controls.

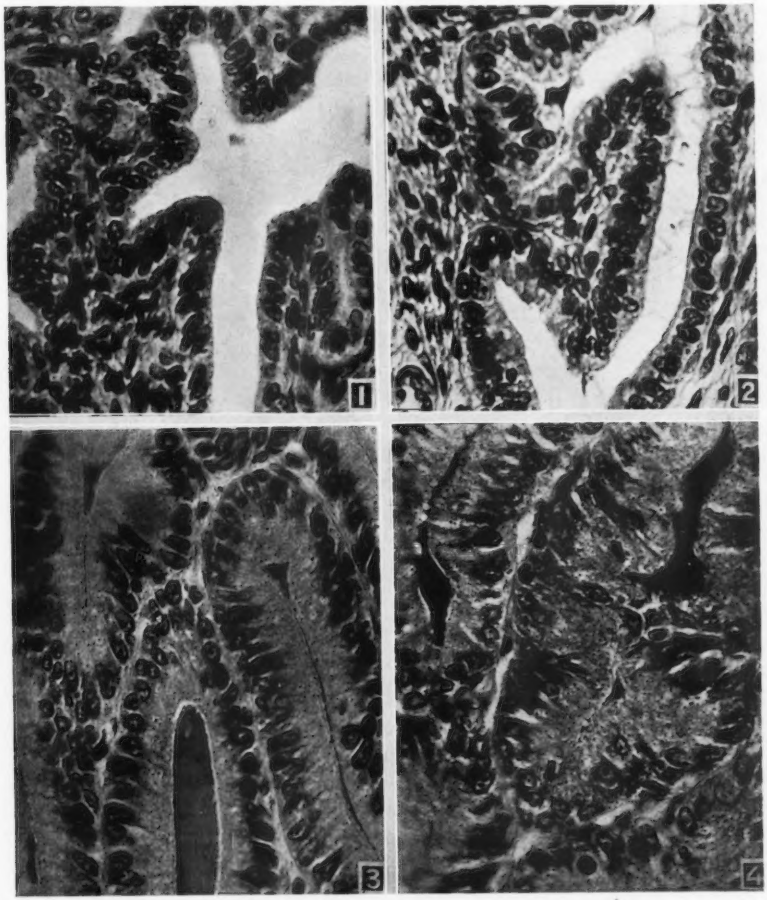
The ventral lobes of the prostate of untreated castrates at this age were similar to those of older castrated males in that involution changes were marked and the epithelium was low and devoid of the diagnostic light areas of secretory cells. Treatment with 0.7 to 0.3 mg. daily however had a decided effect upon this organ and dosages of 0.5 mg. maintained the secretory character at normal control levels. Dosages greater than 0.5 mg. expressed themselves in an increase in actual size of the organ rather than in the secretory character of the epithelium since hypersecretion states are similar in histological appearance to the normal secretory condition.

In fresh weight, 0.5 mg. daily produced a gland approximately the size of the untreated normal control, or even somewhat greater, and larger doses produced larger organs; with 3 mg. daily the organ was some 3 times as large as those of normal littermate controls.

Adults. Large adult males injected daily with androsterone for periods of 20 days required daily dosages of 0.5 mg. to maintain the epithelium of seminal vesicles in a high columnar state with weak secretion granules; such intensity

would be rated histologically slightly below that of the normal control. Dosages of 1 or 2 mg. daily produced histologically normal secretory states in these organs. Doses of 2 mg., however, failed to maintain the fresh weight of seminal vesicles of castrate males at a level characteristic of the normal control.

The normal secretory state of the prostate gland was maintained in the



Photomicrographs of seminal vesicle epithelium. x 560.
1. Castrate control 143, 35 days old. *2.* Normal control 137, 35 days. *3.* Castrate 142, 3 mg. androsterone daily (group 1, table 1). *4.* Rat 115 (table 2), castrated 22nd day, 3 mg. androsterone daily, days 99-117.

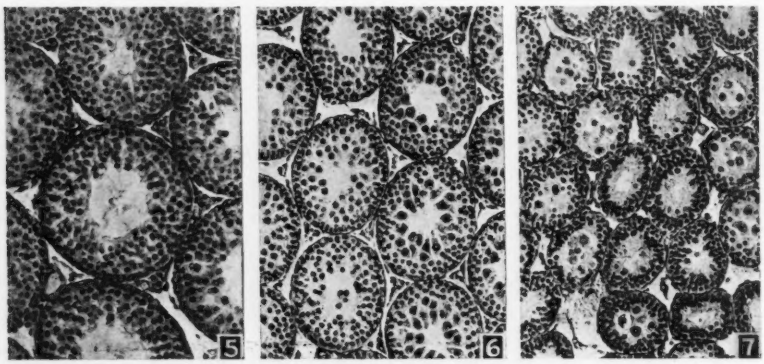
castrate animal after daily doses of 0.3 to 0.5 mg. and similar dosages likewise maintained the size of the gland, expressed by fresh weight, equal to that of the normal control.

In summary, it becomes evident that crystalline androsterone prepared by synthetic means, is able to stimulate the development and function of the

prostate gland and seminal vesicle of the castrate rat at all ages. It is capable of hastening precociously the secretory differentiation and function of the seminal vesicle; in proper doses it maintains the normal growth rate of the accessory reproductive organs in the castrate. In terms of dosage, smaller amounts are required to maintain the normal growth rate at ages up to 35 days than are required at later ages when normal growth is more rapid. It is further evident that the normal secretory state of the organ, as judged by careful study of the cytological picture, can be maintained with smaller daily dosages than are required to maintain the normal growth rate.

B. Treatment Delayed After Testis Removal

In the previous section it was demonstrated that injections beginning immediately after castration maintained normal secretory activity and growth



Photomicrographs of testes from 32 day-old male rats (litter 2, table 3). x 115.
5. Normal untreated control 126. 6. Rat 122, 0.1 mg. androsterone daily, 20 days. 7. Rat 124, 0.3 mg. androsterone daily, 20 days.

of the accessory organs of reproduction. Two litters are cited in table 2 to indicate the reparative effects of androsterone after severe castration-involution changes. These 2 litters were sacrificed at similar ages (118 days) but castration was performed in one group at age 75 days, followed by involutionary changes from a functional state, whereas castration in the other group was prepuberal on day 22; in the latter group therefore the prostate gland would have attained the secretory state but seminal vesicles would not have reached such a state of functional differentiation. Twenty-day injection periods were started 28 and 77 days after castration.

In each group the castrate condition of the accessory organs was returned completely to the normal secretory state within the 20-day period of injection. One mg. daily in the prepuberal castrate group failed to produce the normal secretory epithelium in either the prostate or seminal vesicle but an effect was evident in each. Two and 3 mg. daily produced organs in each series that were indistinguishable microscopically from the organs of any normal adult male. The secretory cells of both prostates and seminal vesicles were normal in ap-

pearance, possessed the characteristic diagnostic elements of normal secreting cells, and were producing secretions entirely characteristic of the normal organs. Figure 4 reveals the normal secretory state of seminal vesicle epithelium of animal 115 castrated on day 22 and receiving daily injections of 3 mg. androsterone from 99-117 days.

By weight the prostate gland and seminal vesicle failed to equal those of the normal littermate control; the prostate weight approached normal weight. It must be remembered, however, that in the normal animal hormone had been supplied constantly by the testicle for a period of 118 days whereas by injection it was supplied for only 20 days. The normal organs are growing rapidly at this age and the amount of androsterone injected was too small to stimulate growth sufficiently rapidly to attain in 20 days a state that had been developing in the controls since birth.

TABLE 2. DELAYED TREATMENT OF CASTRATES WITH ANDROSTERONE.

Rat No.	Treatment	Wt. ventral (post.) prostate, gm.	Wt. (2) sem. ves. gm.	Histological rating	
				prostate	sem. ves.
	Castration, day 75. Daily injections, day 103–118. Sacrifice, day 119.				
158	Cast. control	.0212	.0566	0	0
159	2.0 mg.	.0882	.1100	****	****
160	3.0 mg.	.2128	.1238	****	****
157	Norm. control	.3234	1.1150	****	****
	Castration, day 22. Daily injections, day 99–117. Sacrifice, day 118.				
116	Cast. control	.0028	.0076	0	0
117	Cast. control	.0018	.0050	0	0
118	Cast. control	.0038	.0068	0	0
119	Cast. control	.0036	.0048	0	0
114	1.0 mg.	.0370	.0142	**	*
115	3.0 mg.	.2346	.0926	****	****
120	Norm. control	.4390	.9196	****	****
121	Norm. control	.3494	1.0164	****	****

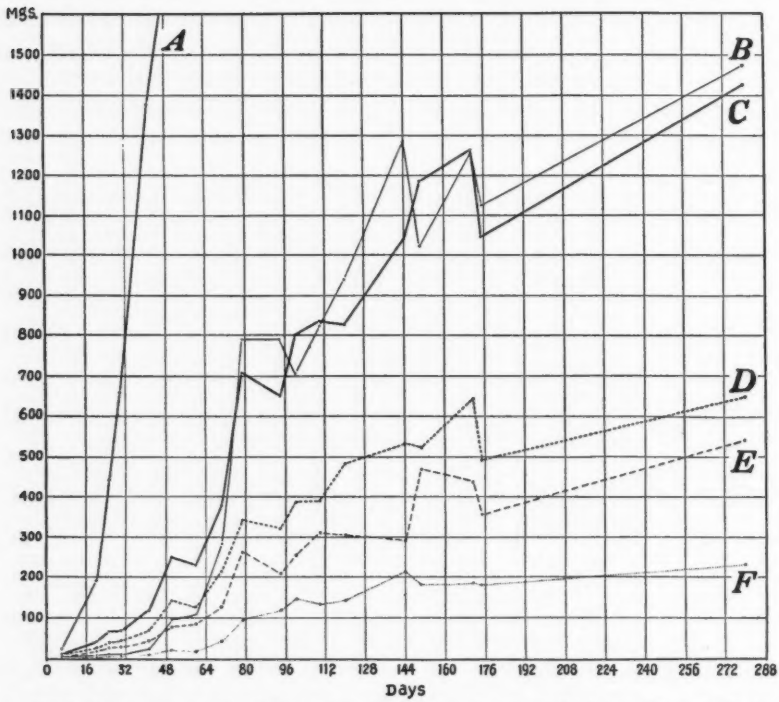
These experiments show that crystalline androsterone alone is able to repair involutional damage caused by testis removal from sexually mature males, as well as from prepuberal animals and to rebuild and differentiate the secretory function of the seminal vesicle and prostate gland to a normal state. The same holds for other portions of the prostate gland as well since the anterior lobes (coagulating glands) reveal similar reparative effects.

C. Prostate-Seminal Vesicle Ratios

One question deserving attention relates to the possible preferential effect of androsterone upon the prostate gland as compared with seminal vesicles. Callow and Deanesly (14) and Deanesly and Parkes (9) discuss the difficulties in establishing a dependable ratio of response of these organs to hormone. We have repeatedly called attention to the differential threshold of these structures and it again becomes apparent in these experiments.

When one examines the prostate seminal vesicle ratio in normal males a great range is revealed. Some of the data obtained from a group of 61 normal

males are presented in the graph. Seminal vesicles, anterior prostate lobes (coagulating glands) and ventral prostate lobes were carefully freed of all fat, under a binocular dissecting microscope, and weighed on a torsion balance immediately after removal. Since the dorsal (middle) lobes cannot be accurately dissected, the entire periurethral complex was cleaned of all fat and weighed as one mass, which included the mid-prostate lobes, ampullary glands, urethra and urethral glands. Ratio values could be established between the seminal vesicles



Graph showing average wt. of tissues from 61 normal male rats sacrificed from 6th to 380th day. Each point on graph determined from average wt. of tissue from 3 to 5 littermate animals. *A.* Wt. of both testes, without epididymis. *B.* Wt. of 2 seminal vesicles. *C.* Wt. total prostate complex (coagulating glands, ventral lobes and mid-lobe with ampullary glands, urethra and urethral glands). *D.* Mid-lobes prostate with ampullary glands, urethra and urethral glands. *E.* Ventral (posterior) prostate lobes. *F.* Anterior prostate (coagulating glands).

and any prostate lobe, but since the response of the entire prostate gland should be reflected the sum of weights of all 3 lobes have constituted the 'prostate complex' here used.

It will be seen from the graph that from the time of establishment of sexual maturity at about 70 days of age the weight of the prostate complex is approximately in a 1 to 1 ratio with seminal vesicles or a 'ratio value' of 1. During prepuberal ages the variation in the ratio values is tremendous and is an expression of several factors. Among these may be mentioned that: *a)* hormone secretion in the testicle develops gradually and slowly; *b)* the pros-

tate gland will respond with growth to an amount of circulating hormone ineffective upon seminal vesicles, and indeed prostate differentiation can apparently continue up to 35 days of age in the absence of testis hormone (13); *c)* increased hormone secretion by the testis occurs about 35 days of age and when the effective threshold for seminal vesicles is reached, their growth, delayed in comparison with the prostate tissue, is greatly stimulated.

When the actual weights are examined for the prostate complex and seminal vesicles the ratio values (prostate/seminal vesicles) at ages 6 to 30 days are from 7 to 9; with the sudden hormone increase at about 35 days the ratio value may reach 14 to 18 since the prostate tissue is growing rapidly at a time when the seminal vesicles are starting to show rapid growth. The ratio values decline gradually as seminal vesicle activity is assumed and at 40 days it is 5; at 60 days of age it drops to 2 and from 70 to 80 days up to 280 days is approximately 1.

If the responses of these organs in a castrated animal to androsterone injections are examined, one finds the ratio value can be varied at will by adjusting the daily dosage. The ratio value characteristic of normal males for similar ages is reached when the amount of androsterone introduced is sufficient to maintain a normal seminal vesicle growth. Thus in males castrated on day 35 and treated with androsterone until day 53 (group 3, table 1), one finds the prostate/seminal-vesicle ratio varies with dosage; the value for normal males is 5. One mg. of androsterone injected daily into castrates produced a ratio value of 20 and the seminal vesicle was but $\frac{1}{3}$ the weight of the normal untreated littermate; 2 mg. of androsterone daily produced a ratio value of 14 and, though seminal vesicles were larger, they were still below that of the normal control male; 3 mg. produced a ratio value of 4.2 (in comparison with 5 for normal controls) and the seminal vesicles were slightly heavier than those of the control.

This shifting of ratio value with dosage is especially marked in prepuberal males (group 1, table 1) since it is approximately at day 35 that an effective hormone level is being established for seminal vesicle growth. In group 1 the ratio values for the 3 normal males was 17, 17 and 12. One mg. daily produced a ratio value in a castrate of 12, and the seminal vesicles were slightly heavier than those of normal controls; 2 mg. produced seminal vesicles 12 times the weight of controls and the prostate/seminal vesicle ratio was reduced to 4.8; 3 mg. producing seminal vesicles 50 times the weight of controls, reduced the ratio value to 1.3 or a value characteristic of adult normal males.

From such an examination, repeated for many litters and under different hormonal conditions, it follows that an amount of androsterone sufficient to provide a normal substitute for testis hormone in the growth of seminal vesicles leads to the production of a prostate/seminal-vesicle ratio characteristic for normal males of the same age; this ratio value varies decidedly in prepuberal ages but is constant after the establishment of full reproductive development. Variation in dosage of androsterone results in variation of ratio values from normal to increased values in lower than maintenance doses,

and from normal to decreased values in overdoses. Thus androsterone fails to show preferential stimulation of the prostate tissue when compared with seminal vesicles and maintains in substitution doses a ratio value characteristic of normal males.

D. Effects of Androsterone in Normal Male Rats

Since a general tendency has existed to believe that gonad hormones would be general reproductive stimulants, special attention has been directed to investigating the effects of crystalline androsterone in the normal male. Moore and Price (15) pointed out that treatments with bull testis extract containing the active principle from the testis prepared by Koch and Gallagher, rather than stimulating the normal young testis, were harmful to it. The suppression of growth and development with actual histological damage was of a character similar to that exerted by estrin on the normal testis and these effects were interpreted not as direct effects upon the testis but as indirect effects by virtue of the suppressive action upon pituitary function. The bull testis extract was not a pure chemical substance hence it becomes of special interest to determine whether crystalline androsterone acts as a testis stimulant, depressant, or is without effect.

Detailed notations for 7 litters are arranged in table 3. Daily dosages of from 0.025 mg. to 6.0 mg. were administered during the 20 days of treatment. Effects can be examined in terms of testis size, modification of spermatogenesis, or of hormone secretion by the testis. The age range of treated animals has included those prior to spermatozoon formation, during this process, shortly after spermatozoon production, young mature males and adults.

Effects on Testis Size. In terms of testis size (table 3, colume '% change over control') normal males treated with androsterone invariably possessed testes lighter in weight than their littermate controls with the single exception of the adult group (245-345 gm. body weight). This reduction in testis weight in treated males ranged from 9 per cent to 88 per cent below the weight of the littermate control rated as unity; where 2 untreated controls were available their average testis weight has been rated unity. Without exception, treated testes have not only been below the average, but in every case under the lowest testis weight of either control male. The decrease in testis weight in each litter was roughly parallel to increased dosages of androsterone, one exception occurring in each of the 57 day and 90 day groups.

There is good evidence that as the animal becomes more mature androsterone is less and less effective on the testis. In the adult group no effect upon the treated testis weight was apparent after a 6.0 mg. daily dose. Further attention will be given to this matter in the discussion.

Effects on Spermatogenesis. Spermatogenetic stimulation if present could be detected by the appearance of sperm-heads in the seminiferous tubules earlier than in untreated controls as pointed out by Moore (32). For such determinations, sacrifice would need to occur at day 30, 31 or 32 since sperm heads appear normally in the testes of this rat colony at 34 to 36 days.

The first 4 litters (table 3) were sacrified on days 31 to 35 to determine whether treated males showed sperm-heads earlier than their littermate controls. Microscopic study of the testes of these 8 normal males and 13 males receiving daily treatment with androsterone in dosages from 0.025 mg. to 1.5 mg. showed that not any of them possessed seminiferous tubules containing sperm-heads. Since testes of normal males at day 36 and older have

TABLE 3. ANDROSTERONE TREATMENT OF NORMAL MALE RATS.

Litter	Rat No.	Treatment		Autopsy			% Change over control	Wt. (2) seminal vesicles. mg.	Ventral prostate mg.	Pituitary implant in mouse	
		Daily dose androsterone mg.	Period, days	Age, days	Body wt. gm.	Wt. (2) testes gm.				ovaries mg.	uterus mg.
1.	145	½ cc. oil	11– 31	32	80	.711		7	35		
	146	½ cc. oil	11– 31	32	80	.783	1	8	40		
	147	0.025	11– 31	32	72	.676	− 9	6	52		
	148	0.05	11– 31	32	72	.674	− 9	7	70		
	149	0.1	11– 31	32	68	.350	−53	6	109		
2.	126			32	68	.700		13	24		
	127			32	65	.584	1	12	34		
	122	0.1	11– 31	32	68	.386	−39	10	66		
	123	0.2	11– 31	32	68	.206	−67	10	105		
	124	0.3	11– 31	32	68	.085	−86	19	120		
	125	0.4	11– 31	32	56	.071	−88	16	94		
3.	109			31	76	.825		18	38		
	110			31	72	.664	1	12	38		
	111	0.5	15– 30	31	84	.608	−18	15	74		
	112	1.0	15– 30	31	80	.540	−27	28	107		
	113	1.5	15– 30	31	84	.240	−67	33	120		
4.	104			35	70	.661		12	33	6.0	40
	105			35	76	.809	1	16	49	4.6	77
	106	0.5	15– 34	35	78	.206	−72	22	117	3.6	19
	107	1.0	15– 34	35	76	.161	−77	22	96	2.9	16
	108	1.5	15– 34	35	80	.134	−81	33	127	4.8	20
										3.0	16 C
5.	76			55	150	1.980	1	94	87		
	77	1.0	35– 54	55	140	1.357	−31	57	163		
	78	2.0	35– 54	55	130	.945	−52	134	256		
6.	86			57	180	2.300	1	214	80	4.6	79
	88	1.0	37– 56	57	135	.783	−65	70	223	2.0	12
	89	2.0	37– 56	57	180	1.700	−26	318	331	3.9	18
	90	3.0	37– 56	57	160	1.710	−26	646		4.6	16
										3.0	18 C
7.	91			90	260	3.050		920	213		
	92			90	270	2.900	1	1093	302		
	93	2.0	70– 89	90	270	1.700	−42	528	238		
	94	4.0	70– 89	90	315	2.540	−14	1590	525		
8.	132			164	245	2.570		1008	281	6.6	20
	133			164	250	2.870	1	574	326	8.4	26
	134			164	270	2.850	1	1091	410	9.6	86
	128	4.0	144–163	164	290	2.940		1950	651	7.6	15
	129	6.0	144–163	164	345	3.110		1210	763	5.8	14
	130	½ cc. oil	144–163	164	265	2.840		940	349	6.0	12
	131	½ cc. oil	144–163	164	295	3.110		1300	460	7.0	13
										C[1] 5.0	6.2

[1] Control, unimplanted littermate.

reached this stage (Moore (32)), it becomes evident that the spermatogenetic process was not hastened to a detectable degree in any of those males receiving treatment. Such treatments would have shown precocious stimulation had this been sufficient to have hastened the attainment of this stage in spermatogenesis by as much as 4 to 5 days.

Testes of normal males at day 55 invariably contain a high percentage of tubules with spermatozoa. In litter 5 the testes of animals 76 and 77 (untreated and 1 mg. daily treatment) each showed 96 per cent of the tubules with sperm-heads or spermatozoa in contrast to the 2 mg. treated male (78)

which had but 12 per cent of the tubules in such a stage of development. In litter 6 the testes of the 1 mg. treated male were more adversely affected than those from the 3 mg. treated male; they containd approximately 3 per cent of tubules with spermatozoa or sperm heads in contrast to others of the litter which contained 80 per cent or more of the tubules in this stage of development.

Testes from larger adult animals (litter 8) failed to show any detectable effect upon the spermatogenetic process even when they received a 6 mg. daily dose. Sufficient androsterone was not available to test further the effects of higher dosages in large adult males.

In the testes of the younger group not only was there a lack of evidence of hastening the spermatogenetic process but on the contrary many of the testes showed positive injury. This was characterized by smaller tubules, fragmentation of cells of the germinal epithelium, and cytologic changes. Tubule diameter determined from averages of cross sections of 25 tubules measured with an ocular micrometer showed that treated testes had tubules from 10 to 45 per cent smaller than normal littermate controls. Little change in tubular diameter occurred in testes of the older treated animals (90 days and adults). Figures 5, 6 and 7 are photomicrographs of testes of the untreated normal, the 0.1 mg. and 0.3 mg. treated males of litter 2 table 3; more severe effects follow higher dosages.

In normal young males, therefore, daily doses of 25 to 100 γ of androsterone failed to suggest any stimulating effect on spermatogenesis; dosages of 1 or 2 mg. administered prior to well developed spermatozoon production (up to 55 days of age) were positively harmful; and dosages up to 6 mg. daily had no perceptible effect upon the testes of adult males.

Effect on Hormone Secretion by the Testis. It is difficult to obtain reliable and convincing data upon the effect of androsterone on the hormone secreting function of the intact testis since the seminal vesicles and prostate gland react both to hormone secreted by the testis and to androsterone. One suggestion however is given in the data of table 3. It will be noted that in litters 5 and 6 the fresh weight of the seminal vesicles of males receiving 1 mg. daily treatment and in litter 7 that receiving 2 mg. treatments were appreciably lower than seminal-vesicle weight of the normal control. This can be interpreted as indicating that the injected androsterone served to decrease hormone secretion by the intact testicle (by virtue of an action upon the pituitary) and that the androsterone injected was insufficient to replace hormone normally provided by the testis. It is only after the 35th day that the testicle normally secretes the hormone in any great amount as shown by the slow growth of the seminal vesicle up to this stage. The testicle can be made to secrete much greater amounts of hormone by treatment with gonadotropic agents such as pregnancy urine or mare serum so that at such ages the seminal vesicles of treated males may show fresh weight increases up to 50 times as compared with normal littermates. The testis-produced-hormone-effect up to this age is, therefore, small as compared with that succeeding the period about 35 days. One mg. doses of androsterone have been shown to have serious

effects upon testis weight and on spermatogenesis up to about 55 days of age, and at the same time have been shown in earlier sections of this report to be insufficient to grow seminal vesicles in the castrate at a rate equal to that which occurs in the normal animal. Two to 3 mg. doses will at such ages about substitute for the testis in maintaining the normal growth rate of seminal vesicles in the castrate hence such dosages in normal animals, though perhaps injurious to hormone secretion by the testis, sufficiently maintain seminal vesicle growth as to make the results incapable of dependable interpretations. It appears well indicated that the prostate and seminal vesicle responses in normal males treated with androsterone cannot be the mere summation of androsterone stimulation and testis hormone stimulation because such data as these indicate that hormone secretion by the testis is hindered by androsterone treatment.

E. Effects of Androsterone on Pituitary Activity

Injections of androgenic substances present in bull testis extract, resulting in decided testis injury in young male rats, was interpreted by Moore and Price as an effect upon the pituitary gland which made unavailable the necessary secretions from the latter organ to maintain testis activity. It was likewise assumed that the pure substance androsterone was perhaps having such an effect. To investigate this possibility the pituitaries from 3 litters of animals of the present series were transplanted into littermate female mice 21 days old and at autopsy 72 hours after implantation weights were taken of the mouse ovaries plus tubes, and the uterus; a single pituitary was transplanted intramuscularly.

The last column of table 3 presents the data obtained. In litter 4 (35 days old) mice receiving the pituitary of an untreated control male rat possessed uteri weighing 2.5 and 4.5 times the uterine weight of the unimplanted control mouse whereas those implanted with pituitaries from rats treated with androsterone had uteri of similar weights to that of the unimplanted control mouse. In litter 6 (57 days) the mouse receiving the pituitary of the untreated male had a uterus that was 4 times the weight of that from the control unimplanted mouse, while those receiving pituitaries from males treated with androsterone gave uterine weights similar to that of the unimplanted control mouse. In the adult male group (litter 8), mice receiving the pituitary from an untreated male (5 cases) had uteri weighing 2 or more times that of the unimplanted control mouse, and the mice receiving the pituitary from an androsterone treated male (2 cases) had uteri that were almost as heavy.

This difference in the pituitaries of androsterone-treated adult males from those of treated younger males parallels the effects upon the testes. Testis injury was evident in the younger groups treated with androsterone whereas larger doses in the mature adults were not injurious. Since the pituitaries of treated adults were not perceptibly less in gonadal stimulating qualities than the untreated ones, the testis injury was not evident in the adult treated males, it suggests that the amount of androsterone administered to

the adult was insufficient to affect the pituitary and lends further credence to the idea that the injurious effect of androsterone upon the testis is by way of the pituitary gland and thus indirect.

It is realized that these data are inadequate to be altogether convincing that androsterone in sufficient amount abolishes the gonadal-stimulating action of the pituitary, and that such an effect is the cause of testis injury, rather than that androsterone has a direct injurious effect on the gonad. They are presented, however, for the suggestive evidence afforded.

DISCUSSION

In addition to the earlier, somewhat brief reports on the effectiveness of androsterone (16, 17, 18, 19) several reports have appeared during the progress of this study (9, 14, 20, 21, 22, 23 24). It has been established clearly that synthetically prepared androsterone and testosterone, as well as some 12 to 18 closely related compounds prepared by Ruzicka and coworkers, show androgenic activity upon injection into the castrated cock or castrated rat (see 9, 22).

For the castrated rat it is well established that androsterone injected immediately following testis removal maintains the normal secretory state of the accessory organs of reproduction, and their weight, when given in sufficiently large daily doses. This dose, however, varies in amount at different ages. Thus 1 mg. daily was more than enough to substitute for the testis between days 15 and 35 but between days 35 and 55 this amount while sufficient to maintain prostate growth and function did not maintain a sufficiently high concentration to provide for normal growth of the seminal vesicles. This fact is an indirect expression of increased testicular function occurring about day 35. Two mg. daily was likewise insufficient for the seminal vesicles whereas 3 mg. was more than a sufficient daily dose. In the castrated adult 2 mg. was also an insufficient dose to maintain the normal growth of the seminal vesicles, which is in keeping with the report of Callow and Deanesly (14) that 2 mg. daily was inadequate but 3 mg. sufficient to maintain the normal weight of these structures.

It is further evident that reparative effects after long time castration follow administration of androsterone and return both prostate and seminal vesicle to normal histological conditions. Whereas in these experiments the weights of the two organs were not returned to weights characteristic of untreated controls within a period of 20 days of injection, it should be pointed out that the largest daily dose employed for this short period (3 mg.) was only a maintenance dose for postpuberal males when injected immediately following testis removal. One could scarcely expect a maintenance dose to so stimulate the organs in a male castrated for 3 months as to grow the accessory organs to a size equal to that attained by normal littermates supplied constantly with the hormone from the intact testes for three months.

A further factor too infrequently appreciated is the different threshold of response for the prostate gland and seminal vesicles. We have repeatedly emphasized from this laboratory the response of the prostate gland to dosages of hormone lower than those required to affect the seminal vesicles (see 25).

In the light of these facts it is not surprising that Callow and Deanesly with 1 mg. androsterone daily (one-third maintenance dose) for 28 days in a rat, castrated prepuberally, and injected only after 2 to 3 months following operation, failed to obtain repair of seminal vesicles whereas prostate glands reacted more favorably.

The contention of several investigators that androsterone shows a preferential stimulation for prostate tissues as contrasted with seminal vesicles (9, 14, 22, 23, 24) has arisen perhaps mainly after the utilization of submaintenance doses. We have emphasized above that the prostate/seminal-vesicle ratio changes during prepuberal development and attains a value of approximately 1.0 at full sexual maturity in comparison with a value of 17 at day 35. It is true that low dosages which will permit prostate tissue to develop, but are too low for seminal vesicle activity, will indicate a selective stimulation for prostate tissue. Thus in our 53 day litter (group 3 table 1) the injection of 1 mg. androsterone daily for 18 days produced a prostate/seminal-vesicle ratio of 20; such a dose is too low for effective growth of seminal vesicles but more than sufficient for prostate growth. In the same litter, 3 mg. daily, a full maintenance dose for seminal vesicles, produced a ratio value of 4 as compared with the ratio value of 5 for the normal control. When, therefore, androsterone is administered in dosages that permit normal growth of seminal vesicles for the respective age no preferential action on prostate tissue is apparent.

Our findings seem to establish clearly that androsterone alone provides for the prostate and seminal vesicles a complete substitute for the hormone secreted by the normal testes. It maintains the secretory state of the organs immediately after castration and repairs castration damage for both prepuberal and postpuberal castration. The substance has been denied possession of estrogenic properties (26) and our own observations show that 3 mg. injections into recently spayed female rats on 3 successive days gave no indications of cornified vaginal cells. In the light of these facts one is unable to agree with Freud (27) that male hormone alone cannot produce normal seminal vesicles in castrate male animals but must depend upon estrogenic substances to act as a 'pacemaker.' Figure 4 indicates the complete reestablishment to normal of the secretory state of seminal vesicles in males castrated prepuberally and treated with pure crystalline androsterone more than 2 months after castration. Injections in this particular case had not restored the weight of the seminal vesicle to that of the normal control which had been continuously exposed to androgenic substances from the testicle but, as discussed above, such a result could not be expected to follow application of this dosage for so short a time. In terms of weight, however, 3 mg. daily prior to day 53 produced seminal vesicles of a weight 3 times those of littermate controls and at day 35 vesicles weighing 50 times the normal control (groups 3 and 1, table 1). From this it appears evident that injection of estrogenic substances is not required to effect maintenance or differentiation of function of the prostate gland or seminal vesicle.

Effects on the Testicle. Generally speaking one must retain the consciousness of possible species differences in responses to any treatment and this

appears to be especially important in the case of the testicle. This study in the rat has failed to reveal any indication of stimulation of the testicle by androsterone either in gross size, spermatogenetic activity or hormonal secretion; the dosage range investigated has fallen between daily administrations of 25 γ and 6 mg. for 20-day periods at ages from 35 days to mature adults. On the contrary, androsterone, as did testis extracts in earlier experiments reported by Moore and Price (15), caused decided injury to the normal testicle in the early growth stages. The injurious effects were gradually less and less as age increased until in mature adult males no injury was apparent with the strongest doses employed. It should be emphasized that 6 mg. daily doses represent twice the amount required to maintain the normal weight of seminal vesicles in the castrate and approximately 6 times that required to maintain the normal secretory differentiation. Similar relative doses of testis extract likewise failed to produce injury to the testes of adult males (15).

A more probable interpretation of these results has not suggested itself since Moore and Price postulated that testicle injury was produced indirectly through an action of the gonad hormone upon the pituitary, which in turn was responsible for the involution changes in the testis. The few litters that could be employed to test pituitary activity in this group show that the pituitary potency in androsterone treatments was reduced to an ineffective level. Upon adults with the largest dosage employed pituitary potency to stimulate the mouse ovary was not abolished.

It is clear therefore that daily treatments of normal male rats during the rapid growth phases of the testis are decidedly injurious to testicular development even in amounts as small as 0.1 mg. It is also true that the smallest quantity of androsterone administered (25 γ daily) not only failed to indicate signs of stimulation but was actually harmful.

Species differences must be emphasized since in another rodent, the annual breeding ground squirrel, androsterone in daily doses of 0.5 to 1.5 mg. gave pronounced testicular responses by increase in size and the earlier appearance of spermatozoa. These experiments are to be reported elsewhere (10).

Among those reporting upon the effects of gonad hormones on the testicle, mention may be made of the findings of Korenchevsky and coworkers who confirmed our findings that androgenic substances materially reduced testicular size in young male rats, and who also reported some reduction in testis weight of injected mature adults (28). Itho and Kon (29) found urinary androgenic substances harmful to growth and development of the testes of young dogs. Ruzicka (30, 31) mentions evidence from Tschopp's experiments that residual testicular nodules in capons were stimulated to further growth by androsterone. The difference in response of the ground squirrel testis to androgenic treatments has not yet received a clear explanation but many other observations on this annual breeding type of mammal indicate a somewhat different hormonal balance from constant breeding types represented in the rat.

SUMMARY

Synthetically prepared androsterone injected in daily subcutaneous doses into castrated rats will: *a)* maintain the secretory function and normal growth

rate of the prostate gland and seminal vesicles; *b)* repair castration damages in prepuberal or postpuberal castrates. Dosage requirements for testicular substitution are lower in 35-day animals than at ages when testicular secretions are greater. In postpuberal animals 0.5 mg. daily is the approximate requirement to maintain normal prostate secretory states and 1 mg. for seminal vesicles. Somewhat less than 3 mg. daily maintains the normal growth rates in seminal vesicles. Threshold responses are important.

The prostate/seminal-vesicle ratio in normal males varies in prepuberal animals, but in sexually mature animals is approximately 1 to 1 (may be 20 in prepuberal males). Androsterone maintains the normal prostate/seminal vesicle ratio in castrates when dosages are sufficient to maintain normal seminal-vesicle growth. The ratio can be shifted at will by varying the dosage. No preferential stimulation of prostate tissue appears with replacing doses.

Androsterone injections into young normal males reduced testicular weights to 12 per cent to 50 per cent those of littermate controls. In mature adults larger doses were not injurious to testicles. Spermatogenesis in rats was not stimulated by androsterone. Pituitaries of androsterone-treated normal males were lower in gonadal stimulating properties than pituitaries of untreated normal controls. Testicular damage by androsterone is believed to be indirect by virtue of its action upon the intact pituitary.

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CHANGES IN THE RELATIVE AMOUNTS OF THE FOLLICLE STIMULATING AND LUTEINIZING HORMONES IN THE HYPOPHYSIS OF THE FEMALE RAT UNDER VARYING EXPERIMENTAL CONDITIONS[1]

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Although the reciprocal relationship between the gonads and the hypophysis has been established, very little has been done to estimate the changes in the follicle stimulating hormone (FSH) and the luteinizing hormone (LH) content of the hypophysis under varying experimental conditions. Chemical separation and direct quantitative determinations of the two sex stimulating components in individual rat hypophyses is not feasible at the present time. However, there is an indirect method available for these measurements which is based on the fact that the size of the ovaries of the immature rat, injected with gonadotropic hormones, is proportional (within certain limits) to the amounts of FSH and LH administered (1). Whenever either of these hormones is the limiting factor in determining the size of the ovaries of the immature rat, in the presence of an excess of the other, an increase in this limiting factor will augment the size of the ovaries. Thus, by injecting constant excess amounts of either FSH or LH into immature female rats, and then giving in addition, constant amounts of hypophyseal tissue from rats under different experimental conditions, the degree of augmentation obtained should be a fair measure of the relative quantities of the two hormones in the glands tested. The absolute quantity of each hormone in the hypophyseal tissue cannot be determined by this method.

The following experiments will show the changes in the relative amounts of FSH and LH which occur in the hypophyses of adult female rats after castration, and of castrated female rats treated with estrin. By a slightly different method, the effect of estrin on the relative amounts of the two sex stimulating hormones in the hypophyses of immature female rats will also be shown.

EXPERIMENTAL

The arrangement of the experiments was essentially similar to that reported by Leonard (2) and Fevold and Hisaw (1) for the 'augmentation reaction.' Immature female rats 26 to 28 days of age were used. To test for the relative amount of the FSH present in the adult rats hypophysis, 50 R.U. of pregnancy urine hormone[2] (LH) was given to one group of immature rats,

[1] Aided by grants from the Rockefeller Foundation.
[2] The pregnancy urine hormone was furnished through the kindness of Dr. J. A. Morrell of Squibb & Co.

3.5 mg. of hypophyseal tissue to be tested was given to a second group of rats, and a combination of both in amounts equal to the above was given to a third group. The percentage of augmentation, calculated by dividing the gain in ovarian weight, induced by the combined preparations, by the sum of the gains obtained from each hormone preparation separately, indicates the relative quantity of the follicle stimulator present in the hypophyseal tissue. The percentage of augmentation must be above 100 to indicate a measurable amount of hormone in the hypophysis. To determine the relative quantity of the LH in the hypophyseal tissue, a constant amount of FSH (menopause urine extract equivalent to 100 cc. per rat) was substituted for PU extract in a procedure otherwise similar to that described above. Three injections were

TABLE 1. OVARIAN WEIGHT INCREASES AND AUGMENTATION SECURED WITH URINARY GONADOTROPIC EXTRACTS AND HYPOPHYSEAL TISSUE.

Group	Hypophyseal Material Ovarian wt. Increase mg.	PU Luteinizer Ovarian wt. Increase mg.	Combination Ovarian wt. Increase mg.	Combination Augmentation %
Normal A.P.	2.3[1]	26.9	31.8	108.8
Castrate A.P.	20.1	26.9	91.5	194.6
Estrin-treated, castrate A.P.	17.8	26.9	51.9	116.1
		FSH, Urine		
Normal A.P.	2.3	26.0	49.6	175.7
Castrate A.P.	20.1	26.0	84.3	182.8
Estrin-treated, castrate A.P.	17.8	26.0	62.7	143.1

[1] Each ovarian weight increase is the average of 4 or more rats. Add 15 mg. to each increase to obtain the actual ovarian weights.

made 24 hours apart; the animals were killed 1 day after the last injection and their ovaries weighed. From 4 to 11 animals were used for each preparation or combination injected.

The donors of the hypophyseal tissue were adult female rats, adult females which had been castrated for three months previously, and similarly castrated rats which had been injected with 10 R.U. of estrin for 10 days before removing the hypophysis. To facilitate administration of the hypophyseal tissue, individual glands were weighed, cut into small pieces in a vial, and dissolved in a 0.1 per cent solution of NaOH in amounts so that 1.5 cc. equalled 3.5 mg. of fresh tissue.

A simpler method of studying the effect of estrin on the relative amounts of FSH and LH in the immature rats' hypophyses was adopted. Instead of following the procedure outlined above, urinary FSH or LH was injected into the estrin-treated and control immature rats directly and the difference in the size of the ovaries produced by the two hormones was noted. If a difference in the size of the ovaries resulted in the estrin-treated and control rats when urinary FSH was injected, then it was assumed that the factor which acts synergistically with FSH in the immature hypophysis was altered which in this case would be the LH. Similarly, if injected urinary LH gave a different degree

of response, then it was assumed that the FSH in the hypophysis was altered by estrin. Ten R.U. of estrin was given daily for varying periods (table 2), and this was followed by injections of either urinary FSH equivalent to 100 cc. of menopause urine or 50 R.U. of pregnancy urine extract (LH).

RESULTS

In the first experiments (table 1), it was noted that the percentage of augmentation increased from 108.8 to 194.6 when combinations of PU extract (LH) with normal and castrated rat hypophyses respectively were used. Following estrin treatment in the castrated female donors, the percentage of augmentation was lowered to 116.1 which was slightly above that found for the normal female donors. Although the percentage of augmentation was markedly lowered after estrin treatment in the castrated females, the gonad stimulating action of the hypophyseal tissue alone was only slightly decreased.

TABLE 2. EFFECT OF ESTRIN ON THE OVARIAN RESPONSE TO URINARY FSH AND LH IN THE IMMATURE FEMALE RAT.

No. of Rats	Days treated with Estrin	Days treated with FSH or LH	Aver. Ovarian weight (mg.) Exp.	Control	Difference %
		Response to FSH			
4	13	4	47.3	52.1	− 9.2
4	13	5	16.4	29.0	−45.0
10	15	6	35.1	65.7	−46.5
9	10	4	52.0	69.2	−24.8
10	22	4	16.1	22.7	−29.0
		Response to LH			
5	11	4	34.6	39.7	−12.8
8	10	4	69.8	61.8	+12.9
12	10	4	69.2	70.1	− 3.3

In contrast to the above, the percentage of augmentation secured by combinations of FSH and hypophyseal tissue from normal and castrated females respectively, increased only slightly (from 175.3 to 182.8). The initial percentage of augmentation was high in this case. Following estrin treatment in the castrated donors, the augmentation was lowered to 143.1 per cent which was below that obtained by combinations of FSH with normal hypophyseal tissue.

When normal immature female rats were given estrin, the ovarian response to injections of FSH and LH was different from that in the controls (table 2). Estrin treatment decreased the ovarian response to injections of FSH by 9.2 to 46.5 per cent. The luteinizing hormone from pregnancy urine, however, produced ovaries of approximately the same size in both estrin-treated and control rats.

DISCUSSION

The results of these experiments show that castration in an adult female rat causes a marked increase in the FSH of the hypophysis but only a slight

increase in the LH.[3] Following estrin treatment in the castrated female rat, the amount of FSH in the hypophysis is depleted to a level slightly above that of the normal gland. Thus there is a differential change in the relative amounts of FSH and LH in the adult female rat hypophysis when the animal is subjected to castration and estrin treatment. Several investigators have previously shown that castration increases the gonad stimulating power of the hypophysis and that estrin treatment decreases the gonad stimulating power of the castrated rats hypophysis. These experiments, however, differentiate the changes in the two known hypophyseal sex stimulating hormones which occur under the same experimental conditions.

The treatment of immature female rats with estrin did not alter the ovarian response to injections of the urinary LH, therefore it is concluded that there is no change in the FSH content of the hypophysis. Equal amounts of estrin injected for the same period decrease the excess FSH in the hypophysis of adult castrated rats. This difference may be explained by the hypertrophy of the hypophysis of normal rats that accompanies estrin treatment (4) which may enable the FSH to remain constant. It may also be possible that the FSH content of the immature rat hypophysis is normally at a level below which it is difficult to cause a further decrease. In this connection it may be mentioned that Lipschutz (5) found an increase in the 'estrogenic effect' of the hypophysis of normal male rats treated with estrin. Fevold, Hisaw and Greep (6) reported a decrease in the FSH of the hypophysis following estrin treatment, which is not in agreement with the results obtained here.

The difference in size of the ovaries of estrin-treated and control immature rats injected with urinary FSH shows that the LH of the hypophysis is depleted. In view of the fact that estrin will decrease the size of the ovaries of the immature rat (7)[4] it might be suggested that this conclusion is unwarranted. Since this effect was not encountered in the previous experiment, it appears unlikely that this explanation will hold. It seems more logical to conclude that the factor in the hypophysis which acts synergistically with FSH is lowered by estrin and that this factor is the LH. This result is in agreement with that of Fevold, Hisaw and Greep (6).

The above methods of determining the relative amounts of FSH and LH in the hypophyses of rats will be helpful in following the quantitative variation of these hormones which presumably occurs in the normal estrous cycle. The differential action of estrin on the two gonad stimulating hormones in normal immature and adult castrated rats indicates that studies must be limited to the normal adult rat hypophysis to further our knowledge of the normal estrous cycle.

SUMMARY

By use of the augmentation reaction, it is possible to follow the changes in the relative amounts of FSH and LH in the adult female rat hypophysis

[3] Since the hypophysis increases in size after castration, the amount of LH in a single gland would be increased. Lipschutz (3) has stated that castration greatly increases the amount of LH in the female hypophysis but not in the male hypophysis.

[4] The effect of estrin in decreasing the size of the ovaries is best demonstrated only when the injections are continued beyond the time of maturity of the rats. This factor is not important in the short estrin treatment in these experiments.

after castration and after estrin treatment in the castrates. It was found that castration greatly increased the FSH but only slightly increased the LH. Estrin treatment in the castrated female lowered the LH level below that found in the normal female but lowered the FSH level to a point just above that of the normal rat. Estrin treatment in immature female rats decreased the response to injected urinary FSH (menopause urine) but not the response to urinary LH (pregnancy urine). The depletion of the LH in the immature rats hypophysis by estrin will account for the decreased response to injections of urinary FSH.

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THE ACTION OF PITUITRIN, ESTRADIOL[1] AND PROGESTERONE ON THE HUMAN UTERUS IN VIVO[2]

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Investigators are constantly presented with the problem whether physiological responses obtained in one species are directly applicable to another. The impression is current that minor variations in response occur in different species, but that the broader generalized reactions are usually applicable from one species to another. In the following discussion we shall present observations that such generalized principles, when applied to certain phases of the physiology of reproduction, are not directly transferable from the rabbit to the human.

The work of Knaus (1), Reynolds and Allen (2, 3), and Corner (4) has established the following facts demonstrating the hormonal control of the rabbit uterus in vivo. *1)* Estrone is necessary for the initiation and maintenance of rhythmic motility of the normal uterus. *2)* The rabbit uterus is refractory to pituitrin when under the influence of a functioning corpus luteum (in pregnancy or pseudopregnancy). *3)* Crude corpus luteum extracts and crystalline progesterone inhibit the estrus mobility and reaction to pituitrin.

On the basis of the first two observations Knaus (1) extended his studies to the human uterus in vivo. His method consisted of the insertion of an elastic bag into the uterus, and the recording of the uterine contractions with a suitable kymograph. His conclusions were as follows. *1)* In women with a healthy reproductive function who menstruate regularly every 28 to 30 days the uterus manifests spontaneous rhythmical contractions during the first 14 days (before ovulation) and reacts positively to posterior lobe pituitary extract during the whole of this period. *2)* Beginning with the 16th day and terminating on the 29th day the uterus loses its capacity for spontaneous contraction and becomes flaccid and sluggish. During this period of immobility the reaction of the uterus to pituitrin is absent (reversed[3]), that is, the uterus fails to contract. *3)* The reason for this absence is that following ovulation a corpus luteum is formed, whose function becomes fully manifest on the 16th day and terminates on the 29th. Progesterone, secreted by the corpus luteum, caused the immobility of the uterus and the reversal to the action of pituitrin. *4)* The appear-

[1] This compound is also known as dihydrotheelin, dihydroestrone, and dihydrofolliculin. It represents a reduction product of estrone and is probably the active hormone in the follicle. It was first prepared by Schwenk and Hildebrandt (Naturwissenschaften, **20**, 658, 1932) and is available commercially as the benzoate, under the name progynon-B.

[2] This work has been supported by a grant from E. R. Squibb & Sons.

[3] The term 'reversed' has crept into the literature when what is actually meant is an absence of contraction. Reversal would imply relaxation, which does not occur.

ance of this reversal signifies that ovulation has taken place within 48 hours. Hence, Knaus used this method for determining the time of ovulation within the menstrual cycle. These observations and deductions of Knaus have led to considerable investigation and discussion. Hermstein (5) agrees with his findings; Shultze (6), Wittenbeck (7) and Tachezy (8) have failed to confirm Knaus' observations.

The problem of corpus luteum physiology assumes, therefore, importance from two viewpoints. First, because Knaus applies it as an exact method of determining the date of ovulation, it becomes one of the main props in support of the theory of a definite 'safe-period.' Second, it poses the question whether the inhibitory action which progesterone exercises upon uterine mobility is applicable to both the human and the rabbit.

We approached this problem by a study of uterine motility in vitro and in vivo. For the experiments in vitro we utilized more than 500 human uteri removed at operation. The following observations were made (9, 10) with the human uterus. *1)* The human uterine strip exhibits spontaneous contractions, and reacts to pituitrin during all phases of the menstrual cycle. *2)* There is no corpus luteum inhibition. The function of the corpus luteum was determined by the menstrual history, inspection of the ovaries in situ in some patients, the examination of the removed ovaries in others, and the examination of the endometrium in all cases.

The observations in vivo were made in the following manner. A small rubber balloon was attached to a no. 12 Keys-Ulzmann cannula. This was then attached to a large and very sensitive recording kymograph.[4] A column of air was the conduction medium between the mercury manometer and the intrauterine bag. The collapsed balloon was gently inserted under sterile precautions into the uterine cavity. If definite bleeding resulted the test was not continued. The system was then filled with air until a pressure of 66 mm. of mercury was obained. Occasionally the patient complained of cramps, when the internal os was passed. These soon stopped. The patient was allowed to remain quietly for about 30 minutes before the observations were begun. She was encouraged to assume a comfortable position. Most patients read while the test was being conducted, an occasional one dozed. Where cooperation was difficult the test was discontinued. Some patients were studied more than once. All injections (pituitary solution, estrone and progesterone) were given intramuscularly in the buttocks. Pituitrin was never injected intravenously as was recommended by Knaus, for in a previous study we noted that such injection may produce mild shock due to peripheral vasospasm. Neither the follicular nor the corpus luteum hormones could be given intravenously for they were oily solutions. An endometrial biopsy was taken at the conclusion of each test, except in a few cases. This endometrial study served as an index of ovarian activity. A total of 50 patients[5] were studied by this method. Only patients with a 28 to 30 day cycle were studied, unless otherwise stated.

[4] We are greatly indebted to Professor Charles C. Lieb for the loan of this very fine instrument. We are also very grateful for his constant encouragement and advice.

[5] The writers are indebted to Miss Belle Francis, nurse in charge of the Gynecological Clinic, for her care and tact in the handling of these patients.

REACTION TO PITUITRIN

Sixteen cases were studied in order to determine the action of pituitrin upon the human uterus in vivo. All phases of the cycle were represented. Each subject received 10 Voegtlein units of pituitary solution (E. R. Squibb & Sons).[6] Only one injection was given. Table 1 and figure 1 record the results. In 7 cases, 2, 3, 8, 9, 10, 11 and 14, a definite premenstrual endometrium existed, hence a corpus luteum was active. These 7 subjects gave a positive response to pituitrin by either a rise in tonus or increased amplitude

TABLE 1. REACTION TO PITUITRIN.

Case	Day of Cycle	Endometrium	Effect on Uterus	Remarks
1	19th	Scanty sample	Contraction	Graph at first flat, good contraction 8 min. later.
2	14th	Early premenstrual	Slight contraction	Definite increase in amplitude.
3	22nd	Premenstrual	Contraction	Increase in amplitude and tonus.
4	19th	Premenstrual	No change	Slight rapid contraction—not definitely changed.
5	11th	Post-menstrual	Contraction	Gradual increase in tonus and amplitude of contractions.
6	5th	Not taken	No reaction	No spontaneous or induced contractions.
7	24th	Scanty sample	Contraction	Increase in tonus and amplitude of contractions.
8	24th	Premenstrual	Contraction	Marked rise in tonus.
9	25th	Premenstrual	Contraction	Gradual rise in tonus. Excursions not increased.
10	27th	Premenstrual	Contraction	Marked rise in tonus.
11	26th	Premenstrual	Contraction	Increase in tonus and amplitude.
12	[1]	Scanty sample	No effect	Addition of progynon-B, 20,000 R.U., later also gave no result.
13	22nd	Scanty sample	Contraction	Graph previously flat. Contractions accelerated by injection.
14	22nd	Premenstrual	Contraction	Graph lost.
15	24th	Early premenstrual	Severe cramps	Graph lost.
16	24th	Scanty sample	No effect	Occasional mild spontaneous contractions.

[1] Amenorrhea for previous 6 months.

of contractions, or both. Spontaneous contractions were present in all of these cases. Pituitrin produced its usual stimulating effect. There was no noticeable corpus luteum effect on the myometrium.

In cases 1, 7 and 13, which also gave a positive response to pituitrin, we did not have enough endometrium in the biopsy to make a positive diagnosis of the menstrual phase, but all occurred in the second half of the menstrual cycle on the 19th, 24th, and 22nd days, respectively. The last 2 cases lie beyond the limits of ovulation, which we place between the 9th and 21st days (11). A corpus luteum effect is therefore to be expected, unless we are dealing with anovulatory cycles. It is conceivable that subject 1, who was examined on the 19th day of a 28-day cycle, had not as yet ovulated, hence the response to pituitrin is that of the postmenstrual phase. This is interesting

[6] We are indebted to Dr. John Anderson and Dr. J. A. Morrell of E. R. Squibb & Sons for the supply of pituitrin and amniotin used in this study.

in view of Knaus' statement (p. 75) that "In women with normal reproductive physiology ovulation always takes place on the 15th day before the onset of the period." This group also does not demonstrate a myometrial inhibition by endogenous progesterone.

Case 5, on the 11th day of the cycle, showed a postmenstrual endometrium, and a positive response to pituitrin. It corresponds therefore to those cases described by Knaus as typical for the postmenstrual phase.

Cases 6 and 12, the former on the 5th day of the cycle and the latter after

TABLE 2. REACTION TO PROGESTERONE.

Case	Day of Cycle	Endometrium	Effect on Uterus	Remarks
20	23rd	Premenstrual	Increased amplitude and tonus	No cramps. Progestin, 2 RB.U.
21	20th	Scanty sample	Increased amplitude	No cramps. Proluton, 1 RB.U.
22	24th	Post-menstrual Anovulatory cycle	Increased amplitude in 10 min.	Rapid contractions throughout. Progestin, 3 RB.U. No cramps.
23	32nd	Scanty sample	Increased amplitude and tonus	First moderate then severe cramps in 30 min. Proluton 2 RB.U.
24	7th	Post-mentrual	Increased amplitude of contractions	Severe cramps after 15 min. Progestin, 2 RB.U. Moderate dysmenorrhea.
25	9th	Post-menstrual	Increased rate after 20 min.	Severe cramps after 25 min. Rare contraction before injection. Proluton 2 RB.U. No dysmen.
26	7th	Scanty sample	No effect. Vomited after 10 min.	Cramp after 30 min. and increased amplitude of contractions. No dysmenorrhea.
27	12th	Post-menstrual	No effect	Spontaneous small rapid contraction. Uterine cramps in 15 min. Progestin 2 cc. Moderate dysmenorrhea.
28	26th	Scanty sample	Increased rate of contraction	Type of contraction changed. Progestin, 2 RB.U. No cramps.
29	23rd	Scanty sample	No effect	Excellent spontaneous contractions. Proluton, 2 RB.U. No cramps.
30	8th	Post-menstrual	Slight increase in amplitude	Uterine cramps in 15 min. Proluton, 2 cc. No dysmenorrhea.
31	4th	No biopsy	No effect	Very severe cramps in 15 min. Spontaneous rapid contractions. No dysmenorrhea.
32	3rd	Premenstrual	No effect	Cramps in 10 min. Proluton, 2 RBU. Slight spontaneous contractions. Moderate dysmenorrhea.
33	11th	Postmenstrual	No effect	Severe cramps after 10 min. Proluton, 2 RB.U. Moderate dysmenorrhea.

6 months amenorrhea, showed no spontaneous contractions nor any reaction to pituitrin. In neither case was an endometrial biopsy obtained. We do not believe that either one had an active corpus luteum at the time the test was taken. Both spontaneous contractions and a positive reaction to pituitrin were therefore to be expected. We (9) had previously noted that occasional human uterine strips in vitro fail to show spontaneous contractions or any pharmacological reactions. Such behavior does not depend upon the phase of the uterine cycle. No satisfactory explanation has been offered.

Case 4 on the 19th day of the cycle and presenting a premenstrual endometrium showed no reaction to pituitrin and only slight rapid spontaneous contractions. Case 16 was tested on the 24th day of the cycle. Insufficient biopsy material was obtained for a definite diagnosis. There were mild infrequent spontaneous contractions and no reaction to pituitrin. Of the entire group of cases these two corresponded most closely to the cases described by Knaus as typical for the premenstrual phase; although the presence of spontaneous contractions does not completely substantiate his views.

Case 15 was tested on the 24th day of the menstrual cycle. The endometrium was of the early premenstrual type. The reaction to pituitrin was characterized by severe uterine cramps that occurred 10 minutes after the injection. We had expected uterine cramps in the other cases of the pituitrin series as well, but none occurred. This is rather surprising when compared with the reaction of the human uterus in situ to progesterone.

REACTION TO PROGESTERONE

The corpus luteum hormone used in this experiment was obtained as proluton (Schering)[7] and progestin (Upjohn).[8] The former is synthetic. The manufacturer's standard of 1 cc. = 1 Rb.U. was found accurate on restandardization by the Clauberg method. Estrone was present in neither preparation. There was no local reaction at the site of injection. The purpose of this study was to note the effect of progesterone on uterine motility, and possibly, the rapidity of absorption from an oily solution. Fourteen cases were examined with the results noted in table 2 (see also fig. 2).

Cases 25, 26, 27, 30, 31, 32 and 33, were studied within the first half of the menstrual cycle. They were all characterized by the fact that uterine cramps occurred within 10 to 15 minutes after the injection of progesterone. The cramps were very severe in some of the cases, and were spontaneously complained of by the patients. It is interesting to note that in cases 26, 27, 30, 31, 32 and 33 the progesterone was without visible effect on the uterus. The modus operandi of this result is difficult to explain. The action could not have involved the entire uterus for the kymograph was sensitive enough to record even very minor changes. On the contrary, spasm of the internal os or isthmus alone could occur without demonstrable change in the graph. It is clinically known that dilatation of the internal os may bring about, in a case of dysmenorrhea, the identical cramps, nausea and vomiting as would be experienced by the patient at the regular menstrual period. Whether the effect of the progesterone is a direct one on the musculature or an indirect one through the autonomic nervous system is not as yet known.

Case 23 presented a similar response on the 32nd day of the cycle. This patient had irregular menstrual periods, with intervals of amenorrhea. Biopsies taken at other times showed the presence of anovulatory cycles and cystic glandular hyperplasia of the endometrium.

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[7] We wish to thank Dr. Gregory Stragnell and Dr. Erwin Schwenk of the Schering Corporation for their very generous supply of proluton and progynon-B.

[8] We are indebted to Dr. George F. Cartland of the Upjohn Company for the progestin used in these experiments.

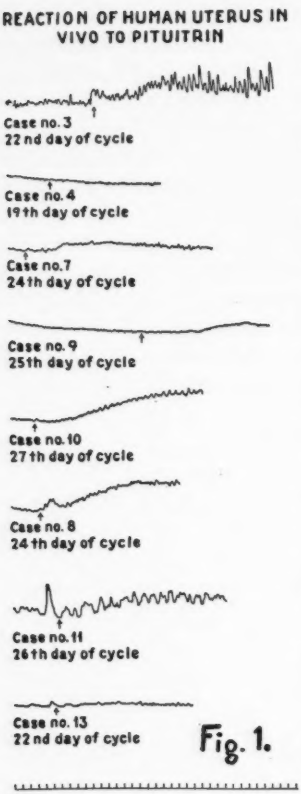

Fig. 1.

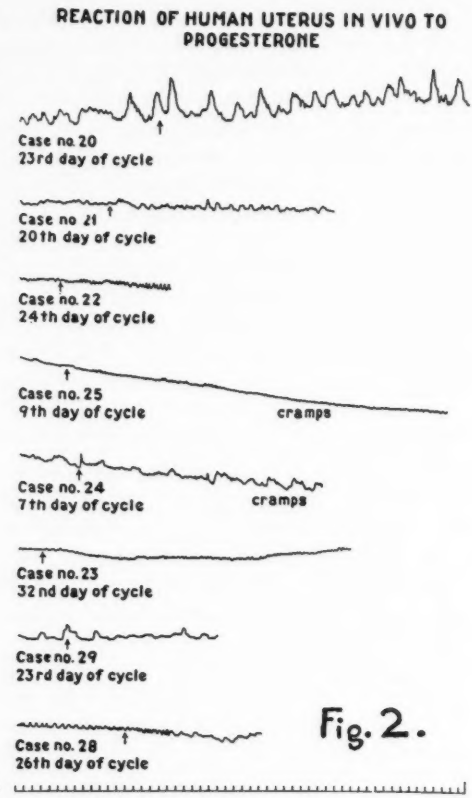

Fig. 2.

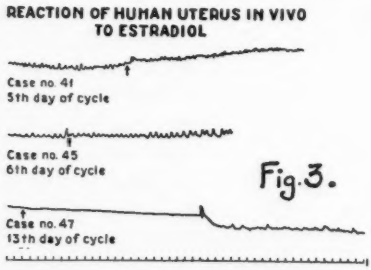
REACTION OF HUMAN UTERUS IN VIVO TO ESTRADIOL
Case no. 41
5th day of cycle
Case no. 45
6th day of cycle
Case no. 47
13th day of cycle

Fig. 3.

In all the graphs the arrow has been placed from 1 to 2 minutes after the actual time of injection, due to the mechanical set-up. All graphs are greatly reduced for purposes of reproduction. Illustration of case 4, figure 1, was included to show our conception of a negative response. There is some increase of rate noticeable in the graph. In case 9, figure 1, the reaction was delayed, possibly because the injection was made into fatty tissue. Time interval—1 minute.

Four of the patients gave a history of moderate cramp-like dysmenorrhea, the remaining 4 had no such symptoms. Therefore, dysmenorrhea does not seem to influence the action of progesterone on the uterus.

The rapidity with which progesterone acts after intramuscular injection is interesting, especially in view of the fact that it is injected in an oily medium.

This rapidity of action is suggestive of an effect on the uterus by way of the nervous system.

Cases 20, 21, 22, 28 and 29 were studied during the second half of the cycle. In the first 4 cases a definite action was obtained on either amplitude, tonus, or rate of contraction. The effect in case 20 was startling in its intensity. In spite of this change in uterine motility no uterine cramps were complained of. Case 22 frequently presented anovulatory cycles. We cannot offer a satisfactory reason why progesterone should produce cramps in the first half of the cycle and not in the second. The extent of uterine response was not a factor, nor did the form of the graph show any characteristic change. It would seem that the injection of 2 Rb.U. of progesterone might be used to determine the time of ovulation.

TABLE 3. REACTION TO ESTRADIOL (BENZOATE).

Case	Day of Cycle	Endometrium	Effect on Uterus	Remarks
40	17th	Insufficient Tissue	None	No cramps.
41	[1]	Cystic dilatation of glands	Contraction Increase in tonus	Fröhlich. Cramps in 10 min.
42	12th	Insufficient Tissue	No effect	Spontaneous rapid contractions. No cramps.
43	6th	Insufficient Tissue	Slight increase in tonus	Cramps in 10 min.
44	18th	Slight cystic dilatation Transitional	No effect	No cramps. Slow spontaneous contractions.
45	6th	Postmenstrual	Slight increase in amplitude, then spasm	Moderate cramps 20 min. later coinciding with period of spasm.
46	11th		No effect	Rapid contractions of small amplitude. Cramps in 20 min.
47	13th	Postmenstrual	Contractions in 20 min.	No spontaneous contractions, no cramps.
48	5th	No biopsy	No effect	No spontaneous contractions, none induced by pituitrin. No cramps.

[1] Last cycle 5 months previously.

In view of the fact that progesterone may elicit cramps during the first half of the cycle and changes in uterine motility in the second, the question arises as to how safe is the injection of corpus luteum hormone, when this is administered very early in pregnancy for the prevention of habitual abortion. Numerous reports have appeared which testify to this therapeutic effect. Our experience has not been completely gratifying, especially when bleeding has occurred. On a number of occasions the injection of progesterone has been accompanied by cramps and spurts of renewed bleeding. Possibly the therapeutic effect of progesterone on threatened abortion depends on some factor other than uterine motility, possibly on the decidua. Or again, the action of progesterone may be different during pregnancy, and the findings during the menstrual cycle may not be applicable. Further studies, by means of the intrauterine bag, during early pregnancy are essential. The studies of Rucker (12) on the uterus in labor and those of Moir (13) and Adair and Davis (14) on the postpartum uterus are not directly applicable.

REACTION TO ESTRADIOL (*benzoate*)

The action of estrone on the human uterus in vitro has not been satisfactorily studied because of the low solubility of the hormone in water. The patients studied were each injected with 20,000 R.U. of estradiol benzoate (progynon-B)[1, 7] intramuscularly in the buttocks. The results were not marked. Only case 41 responded by a significant contraction. Four of the cases had cramps, but the extent of the uterine reaction did not run parallel with the severity of the cramps. For instance, case 41 had the greatest response but the cramps were only moderate, while case 46 had no reaction but had moderate cramps. As in the experiments with progesterone the cause of the cramps is not quite clear. In case 45 the cramps coincided with a period of spasm on the graph. The phase of the cycle seemed without effect, though the cases were nearly all in the postmenstrual phase. The onset of cramps occurred after 10 to 20 minutes. This would imply rapid absorption from an oily solution and, probably, the breaking down of the dihydroestrone benzoate into dihydroestrone and benzoic acid. (See table 3 and fig. 3.)

SUMMARY

Experiments on human uteri in vivo demonstrate that the uterus shows spontaneous contractions during all phases of the menstrual cycle. The uterus reacts to pituitrin in a positive manner during all phases of the cycle. There is no corpus luteum inhibition, hence the reaction offers no evidence as to the time of ovulation.

Progesterone may occasionally produce an increase in tonus and amplitude of contraction. Progesterone produces uterine cramps during the postmenstrual phase of the cycle, but not during the premenstrual phase. The cramps are not associated with significant changes in the graph nor with a previous history of dysmenorrhea.

Estradiol (benzoate) occasionally produces a rise in amplitude and tonus. Estradiol (benzoate) may produce cramps during the first half of the cycle, without significant changes in the tracing.

Oily solutions of progesterone and estradiol benzoate are rapidly absorbed from the local site of injection, the effects being observed within 30 minutes.

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COMPARATIVE ACTION OF GONAD-STIMULATING HORMONES ON THE OVARIES OF RATS[1]

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We have previously reported that the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH) of the pituitary work together in producing ovarian development (1). When they are injected separately, the LH does not increase the weight of the ovaries of immature rats while the FSH produces moderate ovarian development due to the growth of noncystic follicles. Evans et al (2) have shown that the action of the gonad-stimulating substance obtained from pregnancy urine (PU) is limited in its action on immature rat ovaries, in that ovarian weight increases with increased dosage up to a certain point beyond which additional hormone has little effect.

We have prepared a follicle-stimulating preparation from the anterior lobe of the pituitary which is free from adrenotropic, thyrotropic, growth, lactogenic and luteinizing factors as far as can be determined by the usual tests for these substances. The action of this preparation on the ovaries of rats is rather sharply limited in that it is difficult to produce ovaries beyond a certain weight. This is true to a certain extent in normal immature animals but more definitely so in hypophysectomized rats, as the following results will demonstrate.

The experiments were carried out on normal rats 21-days old at the start of the experiment and on rats which were hypophysectomized when 24-days old. The animals were injected for 5 days, twice daily, killed the morning of the sixth day and the ovaries weighed and examined.

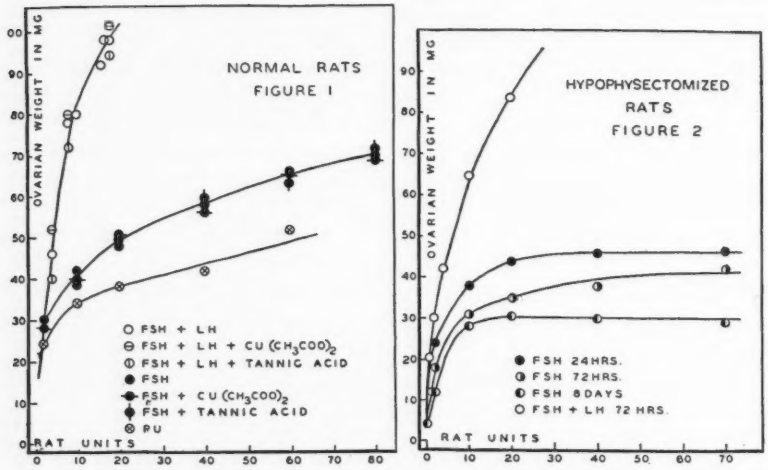
When FSH is injected into normal 21-day old rats in graded doses, the ovaries increase in size roughly in proportion to the dosage employed until they reach a weight of 45 to 50 mg. From that point on very little additional development takes place when the dosage is raised and an approximate limit is reached at an ovarian weight of about 70 mg. (fig. 1). However the curve representing the relationship between weight and dosage does not become perfectly horizontal indicating that there is perhaps no sharp limit to which the ovaries may be grown with FSH in normal immature rats. This is of course to be expected since the estrin which is elaborated by the developing follicles causes the pituitary to secrete LH which would result in some augmentation (3). It is known also that if FSH injections are continued for longer periods than those used in these experiments, augmentation and luteinization results.

When FSH and LH are injected together the results are quite different in that the ovarian weights increase regularly with the dosage (fig. 1). Thus,

[1] Aided in part by a grant from the Rockefeller Foundation.

while a rat unit of FSH is equivalent to a rat unit of FSH plus LH in that both are taken as that amount of the preparation necessary to increase the ovarian weight 50 to 100 per cent, 10 R.U. of FSH produce ovaries weighing 42 mg., while the ovarian weights of the animals receiving 10 R.U. of FSH plus LH are 80 mg. By increasing the dosage, ovaries weighing as much as 600 mg. have been obtained, which fact demonstrates that the action of the two hormones is not limited as is the FSH alone.

The action of tannic acid and copper salts in augmenting the effect of FSH and FSH plus LH has been reported (3, 4). It was of interest to determine if these substances altered the limits of the action of FSH or of FSH plus LH. In the experiments with copper acetate the rats were given a combination of the



gonadotropic preparations plus 1 mg. of the copper salt. When tannic acid was used the resulting precipitate was washed to remove excess acid and injected in aqueous emulsion. The results were again plotted on the basis of rat units of the combinations. As is seen from the curves, the results for FSH alone, FSH plus copper acetate or of tannated FSH were practically identical. The same was true of FSH plus LH, FSH plus LH plus copper acetate and for FSH plus LH tannated. It is thus apparent that these substances probably do not produce their effects by modifying the end organ but in some way regulate the rate and degree of utilization of the hormones.

While it has previously been demonstrated that PU is limited in its action on the female rat ovary, we determined its quantitative action on the rats from our colony. The response of the ovaries to this substance was more gradual than for FSH but the ovaries did increase with increasing dosage and reached approximately 70 mg. when 200 R.U. were given. Here again there seems to be no sharp upper limit though it becomes increasingly difficult to increase the size of the ovaries.

The results for hypophysectomized rats demonstrated more markedly the

limitations of FSH alone in producing ovarian development. Three groups of animals were used; the first consisted of rats which had been hypophysectomized for one day before beginning the injections, in the second the animals were hypophysectomized for 3 days and in the third they were operated upon 8 days before the experiment was started. In each group the limit of ovarian development was reached rather quickly and additional hormone had no further effect. Also it is significant to note that the upper limit decreased with increasing length of hypophysectomy which is undoubtedly due to the progressive atrophy of the ovaries following removal of the pituitary (fig. 2).

No curves are given for LH since we have shown that LH, even in large doses, does not increase the weight of the ovaries of immature rats when injected for 5 days. In hypophysectomized rats, injections of LH for 10 or 20 days does not result in ovarian enlargement.

When FSH and LH were injected together into hypophysectomized females the results were comparable to those obtained when the same preparations were injected into normal females. The response was quite proportional to the dosage and the increase was much greater than with FSH alone.

One of the facts which militated against the idea of a duality of the gonadotropic hormones was that luteinization always took place in the ovaries of normal, immature rats if injections of FSH were continued longer than 5 or 6 days (5). However in hypophysectomized rats we have found it possible to inject the 'pure' FSH for 10 or 20 days without luteinization of the follicles. The luteinization which occurs when normal rats are used is evidently due to the action of estrin, secreted by the developing follicles, on the pituitary causing it to release LH as first shown by Hohlweg (6). It is therefore an indirect effect which cannot take place in a pituitaryless rat.

SUMMARY

The facts which have been presented offer additional evidence for the duality of the pituitary gonadotropic hormones. The two preparations affect the ovary in a characteristic manner, not only from a qualitative standpoint as has been reported but also the quantitative limits of the wo singly and when combined are quite different. The action of FSH is quite sharply limited in its effect while that of FSH plus LH is not.

The results further prove that tannic acid and copper salts do not alter the nature of the ovarian response to gonadotropic preparations in that the quantitative limits of the physiological reaction are not changed. This fact again differentiates the increased action due to these substances from the augmentation of FSH by the LH preparation.

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THE EFFECT OF COMPLETE AND PARTIAL HYPOPHYSECTOMY IN ADULT ALBINO RATS ON WATER, CHLORIDE, SODIUM, POTASSIUM AND SULFUR METABOLISM

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In a previous paper (1) the effect of complete and partial hypophysectomy in adult albino rats on nitrogen, calcium and phosphorus metabolism was reported. In this communication changes in water, chloride, sodium, potassium and sulfur metabolism will be presented.

EXPERIMENTAL

Eleven male and 10 female albino rats (Wistar strain) were used in the experiments. At the time of hypophysectomy they were about 3 months of age. The rats were kept in groups of 3 or 4 in metabolism cages as described in a previous publication (2). Urine and feces were collected twice a week. The urine was analyzed for total sulfur (3), total and inorganic sulfates (Folin), sodium and potassium (4) and chloride (modified Volhard-Harvey method). Feces were analyzed for total sulfur (5) and chloride (open Carius method). Food was analyzed for total sulfur, sodium, potassium and chloride. The rats were studied for a control period of 4 weeks before, and 3 periods of 3, 2 and 5 weeks after hypophysectomy. Three days were allowed for recovery after operation before the excreta were collected again. The data are presented in tables 1 and 2.

Water Metabolism. In the male rats, water intake increased from 17 cc. daily during the control period to 46 cc. during the first 3 weeks after hypophysectomy. During the 4th and 5th week it dropped to 38 cc. After that it dropped to 29 cc. where it remained for the duration of the experiment (10 weeks after operation). In the female rats the intake returned to normal after 5 weeks. Urinary water excretion in the males increased from 14 per cent of the intake during the control period to 54 per cent during the first 3 weeks, dropped to 48 per cent during the 4th and 5th week and remained at 46 per cent for 10 weeks after operation. In the females the urine volume returned to normal after 5 weeks.

In the partially hypophysectomized male rats the water intake rose slightly, from 21 cc. to 27 cc., during the first 3 weeks after operation. The urine volume rose from 16 per cent to 27 per cent of the water intake. After that, intake and output returned to normal.

Chloride Metabolism. In the male rats urinary chloride excretion—though there was a slight drop in the absolute values—rose from 43 per cent of the intake during the control period to 86 per cent during the first 3 weeks after hypophysectomy. It decreased to 84 per cent during the 4th and 5th week and remained at 68 per cent for 10 weeks after operation. In the females the rise was neither as pronounced nor of such long duration. Urinary chloride excretion increased from 51 per cent of the intake during the control period to 68 per cent during the first 3 weeks. It dropped to normal during the 4th and 5th week—absolute values being cut in half—and to below normal—33 per cent—during the 6th to 10th week. In the partially hypophysectomized males there

TABLE I. WATER, CHLORIDE, SODIUM AND POTASSIUM METABOLISM IN RATS BEFORE AND AFTER HYPOPHYSECTOMY. DAILY AVERAGES BY PERIODS.

Date 1935–36	Urine vol. cc.	Water Intake cc.	Chloride, as mg. Cl					Sodium, mg.			Potassium, mg.			pH
			Urine	Feces	Total Excret.	Intake	Retention	Urinary Excret.	Intake	Retention	Urinary Excret.	Intake	Retention	
A. Male rats, before and after complete hypophysectomy														
Oct. 29–Nov. 25	2.3	17	49.9	15.3	65.2	115.0	49.8	30.8	71.9	41.1	23.6	55.8	32.2	7.6
*Nov. 30–Dec. 20	24.9	46	47.1	2.6	49.7	54.3	4.6	29.5	34.2	4.7	23.9	26.5	2.6	8.2
Dec. 21–Jan. 3	18.2	38	42.6	1.3	43.9	50.7	6.8	25.5	31.6	6.1	17.8	24.5	6.7	7.8
Jan. 4–Feb. 7	13.2	29	37.1	2.0	39.1	54.8	15.7	24.1	34.2	10.1	17.1	26.6	9.5	8.1
B. Female rats, before and after complete hypophysectomy														
Oct. 29–Nov. 25	3.1	18	50.4	9.5	59.9	99.6	39.7	31.6	62.1	30.5	23.3	48.3	25.0	8.3
*Nov. 30–Dec. 20	14.1	37	45.9	2.9	48.8	67.0	18.2	30.6	41.8	11.2	21.3	32.9	11.6	8.3
Dec. 21–Jan. 3	5.2	23	25.2	1.9	27.1	49.8	22.7	16.0	30.8	14.8	11.1	24.1	13.0	8.3
an. 4–Feb. 7	1.7	19	19.9	0.8	20.7	60.1	39.4	13.4	37.5	24.1	7.5	29.2	21.7	6.5
C. Male rats, before and after partial hypophysectomy														
Oct. 29–Nov. 25	3.4	21	65.6	13.7	79.3	127.9	48.6	43.0	79.9	36.9	30.6	62.1	31.5	8.3
†Nov. 30–Dec. 20	7.3	27	53.5	3.5	57.0	100.2	43.2	34.3	62.6	28.3	23.7	48.6	24.9	7.3
Dec. 21–Jan. 3	1.5	22	58.9	3.2	62.1	105.2	43.1	35.7	65.7	30.0	24.7	51.0	26.3	6.8
Jan. 4–Feb. 7	3.9	25	60.6	2.5	63.1	124.3	61.2	38.5	77.5	39.0	25.4	60.2	34.8	6.8

* Hypophysectomized November 26th.
† Partial hypophysectomy November 26th.

was very little change in the urinary excretion. Fecal chloride excretion dropped with the diminished intake from about 13 per cent of the intake during the control period to about 4 per cent after operation in all animals. Excretion by way of the gut, which during the control period amounted to 23 per cent of the total excretion in the males and to 16 per cent in the females, dropped to about 5 per cent after operation. In the partially hypophysectomized rats fecal excretion also dropped from 17 per cent of the total excretion to about 5 per cent, in spite of the fact that the intake returned to normal 5 weeks after operation.

Sodium and Potassium Metabolism. The urinary soduim and potassium excretion followed closely that of the chloride ion; expressed in percentage of intake it was practically the same as that of chloride.

Sulfur Metabolism. In the male rats total urinary sulfur excretion rose during the first 5 weeks after hypophysectomy from 17 per cent of the intake during the control period to 48 per cent, and was still 36 per cent 10 weeks after operation. The excretion of inorganic sulfates, expressed in percentage of

the total urinary sulfur excretion rose from 66 per cent during the control period to 71 per cent during the first 3 weeks after operation. After that it returned to normal. Neutral sulfur excretion, expressed in percentage of total urinary sulfur excretion rose from 26 per cent during the control period to 30 per cent after a slight initial drop. The percentage of the sulfur intake which was excreted by way of the gut was unchanged, though the absolute values dropped with the diminished intake, which decreased to about half of that consumed during the control period. This drop in intake, combined with the increased urinary sulfur excretion accounted for a decrease in retention from 58

TABLE 2. SULFUR METABOLISM IN RATS BEFORE AND AFTER HYPOPHYSECTOMY. DAILY AVERAGES BY PERIODS.

Date 1935–36	Total Sulfur, in mg.					Neutral S mg.	Inorganic Sulfates mg.	Ethereal Sulfates mg.
	Urine	Feces	Total Excret.	Intake	Retention			
A. Male rats, before and after complete hypophysectomy								
Oct. 29–Nov. 25	6.5	9.4	15.9	37.5	21.6	1.7	4.3	0.5
*Nov. 30–Dec. 20	7.9	4.2	12.1	17.7	5.6	1.9	5.6	0.4
Dec. 21–Jan. 3	7.9	4.1	12.0	16.5	4.5	2.4	5.1	0.4
Jan. 4–Feb. 7	6.4	4.4	10.8	17.7	6.9	1.9	4.3	0.2
B. Female rats, before and after complete hypophysectomy								
Oct. 29–Nov. 25	7.6	8.1	15.7	32.4	16.7	1.8	5.4	0.4
*Nov. 30–Dec. 20	7.0	5.5	12.5	21.9	9.4	1.4	5.4	0.2
Dec. 21–Jan. 3	4.6	3.9	8.5	16.2	7.7	1.3	3.1	0.2
Jan. 4–Feb. 7	2.9	4.9	7.8	19.5	11.7	1.2	1.6	0.1
C. Male rats, before and after partial hypophysectomy								
Oct. 29–Nov. 25	8.8	10.4	19.2	41.7	22.5	2.5	5.6	0.7
†Nov. 30–Dec. 20	7.6	8.2	15.8	32.7	16.9	2.0	5.3	0.3
Dec. 21–Jan. 3	7.2	8.5	15.7	34.2	18.5	2.1	4.7	0.4
Jan. 4–Feb. 7	9.1	10.1	19.2	40.5	21.3	2.8	5.8	0.5

* Hypophysectomy November 26th.
† Partial hypophysectomy November 26th.

per cent of the intake during the control period to 30 per cent during the first 5 weeks after operation. During the following 5 weeks there was a slight increase in retention to 39 per cent of the intake.

In the female rats total urinary sulfur excretion rose from 23 per cent of the intake during the control period to 32 per cent during the first 3 weeks after operation. It dropped to 28 per cent during the 4th and 5th week and to 15 per cent during the 6th to 10th week.

The excretion of inorganic sulfates, expressed in percentage of total urinary sulfur excretion, rose from 71 per cent during the control period to 77 per cent during the first 3 weeks after operation. It then dropped progressively to 67 per cent during the 4th and 5th week and to 55 per cent during the 6th to 10th week. Neutral sulfur excretion expressed in percentage of total urinary sulfur excretion rose from 24 per cent to 40 per cent after a slight initial drop. The

fecal sulfur excretion in percentage of intake remained unchanged. Retention in percentage of intake dropped from 52 per cent during the control period to 43 per cent during the first 3 weeks after operation, then rose, in spite of the decreased intake, till it reached 65 per cent during the 6th to 10th week after hypophysectomy. In the partially hypophysectomized male rats there was hardly any change in sulfur metabolism.

DISCUSSION

It has been shown as early as 1913 (6) that the polyuria of diabetes insipidus can be controlled by injections of extracts of the hypophysis. Since that time a great deal of work has been done on the relation of the hypophysis to water metabolism, but the results of different observers do not show complete agreement. Downs and Geiling (7) conclude from their experiments on mice which contain 6 to 8 per cent more water than the controls if injected with an alkaline extract of the hypophysis, that a water-balance principle is present in the anterior lobe of the hypophysis. Pencharz and his coworkers (8) state that complete hypophysectomy is followed by transient polydipsia and polyuria which last 24 to 36 hours. Richter (9) found a much longer duration of the diabetes insipidus, but stresses the fact that polydipsia and polyuria are never permanent if both lobes of the hypophysis are removed. On the basis of his experiments, polydipsia and polyuria would be expressions of a disturbance of the balance between the anti-diuretic pituitrin from the posterior lobe and the diuretic agent from the anterior lobe. Newton and Smirk (10) believe that the hypophysis and the hypothalamus are not indispensable parts of the mechanism responsible for controlling water diuresis. They suggest that, provided the kidneys are still capable of a water diuresis, some physiological mechanism not involving the hypophyseal hormone can restore the rate of urine formation to normal after spontaneous polyuria has developed. They quote experiments in which an excess of water was given to hypophysectomized animals in support of their contention that the diminution in the rate of urine flow as excess of water is excreted is to be taken as evidence that a natural control of diuresis is present which responds to the needs of the organism. This ability to diminish the rate of urine formation is unconnected with the secretory activity of the hypophysis, since this has been entirely removed. In our experiments polydipsia and polyuria returned to normal after 5 weeks in the female as well as in the partially hypophysectomized male rats. In the completely hypophysectomized males, however, an increase of 59 per cent in the water intake over that during the control period, and a corresponding polyuria, were still present 10 weeks after operation. While the hypophysis is doubtless involved in the regulation of water metabolism, a compensatory mechanism seems to develop after a shorter or longer interval in the absence of the gland.

Contrary to our findings in suprarenalectomized rats, in which the chloride retention in percentage of intake stayed the same after operation as during the control period, retention in the male rats dropped considerably. This was not due to a simple washing out of the chlorides by the pronounced diuresis,

since the absolute amounts excreted by the kidneys showed little variation, regardless of the fact that the intake decreased by half. In the females adjustment took place 3 weeks after operation.

The absolute values of urinary sodium and potassium excretion were the same during the first 3 weeks after hypophysectomy as during the control period. But since the intake dropped to about half the former amount, excretion in percentage of intake rose to the same high level as that of chloride. After this excretion decreased somewhat in the males, but not sufficiently to bring retention in percentage of intake back to normal levels, while in the females adjustment to normal was reached. This inability to retain sufficient potassium agrees with the finding by Marenzi (11) of a decrease in potassium in the blood plasma of hypophysectomized dogs.

The disturbance in sulfur metabolism, encountered in hypophysectomized rats is probably attributable to the lack of adrenotropic hormone which prevents the proper functioning of the suprarenals. Just as in suprarenalectomized animals (12), we found an increase in urinary sulfur excretion. The proportion of neutral sulfur excreted also rose, though to a lesser degree than after suprarenalectomy. This indicates the same type of disturbance in the endogenous sulfur metabolism as in suprarenalectomized animals, suggesting that the hypophysis controls sulfur metabolism by way of the suprarenals.

SUMMARY

The water, chloride, sodium, potassium and sulfur metabolism of completely and partially hypophysectomized rats was studied. In the male rats pronounced polydipsia and polyuria occurred after hypophysectomy, which was still present 10 weeks after operation. In the female rats water intake and output returned to normal after 5 weeks. In the partially hypophysectomized animals the rise was much less pronounced and persisted only for 3 weeks. Urinary chloride excretion in the male rats rose from 43 per cent of the intake during the control period to 86 per cent during the first 3 weeks after hypophysectomy, and was 68 per cent 10 weeks after operation. In the females the increase, from 51 per cent of the intake to 68 per cent, lasted only for 3 weeks. It dropped to normal during the next 2 weeks, and even to below normal—33 per cent—during the 6th to 10th week after operation. In the partially hypophysectomized males there was little change in the urinary excretion. Fecal chloride excretion dropped with the diminished intake in all animals. The urinary sodium and potassium excretion followed closely that of the chloride ion; expressed in percentage of intake it was practically the same as that of chloride. In the male rats total urinary sulfur excretion rose from 17 per cent of the intake during the control period to 48 per cent during the first 5 weeks after hypophysectomy, and was 36 per cent 10 weeks after operation. The rise in the excretion of inorganic sulfates, expressed in percentage of the total urinary sulfur excretion returned to normal after 3 weeks. Neutral sulfur excretion increased. The percentage of the fecal sulfur excretion was unchanged, though absolute values fell with the diminished intake. In the female rats the excretion of total urinary sulfur and of inorganic sulfates

dropped to normal 3 weeks after operation and to below normal after that. Neutral sulfur excretion was increased; fecal sulfur excretion remained unchanged. In the partially hypophysectomized rats there was hardly any change in sulfur metabolism.

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THE METABOLISM OF WATER, CHLORIDE, POTASSIUM, SODIUM, CALCIUM, MAGNESIUM AND PHOSPHORUS IN SUPRARENALECTOMIZED RATS

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The studies on the metabolism of water, chloride, potassium, sodium, calcium, magnesium and phosphorus presented in this communication form part of an extended investigation of the metabolic changes taking place in animals deprived of one or the other of the endocrine glands.

EXPERIMENTAL

Twenty-four male and female albino rats (Wistar strain) were used in the experiment. At the time of suprarenalectomy they were about 3 months of age. The rats were kept in groups of 4 in metabolism cages, as described in a previous publication (1). The diet was the same as the one we had found successful in a former study on suprarenalectomized rats (2). Water, redistilled from glass, was provided from automatic glass fountains during the control period, and the first period after suprarenalectomy. During the second period after operation this was replaced by a 0.5 per cent NaCl solution, prepared from the pure salt.

Urine and feces were collected twice a week. The urine was analyzed for chloride (modified Volhard-Harvey method), sodium and potassium (3), calcium (volumetric), magnesium (4), and phosphorus (Fiske-Subbarow). Feces were analyzed for chloride (open Carius method), calcium, magnesium (5) and phosphorus. Food was analyzed for chloride, sodium, potassium, calcium, magnesium and phosphorus.

The rats were studied for a control period of 4 weeks before, and 2 periods of 3 weeks each after suprarenalectomy. Three days were allowed for recovery after operation before the excreta were collected again. No macroscopic suprarenal tissues were found at autopsy. The figures mentioned in the discussion are averages of the results obtained on all the animals. A typical protocol is presented in the tables, showing daily averages for one rat.

DISCUSSION

Harrop and coworkers (6) state that the loss of water, in the absence of the cortical hormone, is sustained partly by the blood plasma, but to a far greater extent by the interstitial body fluid. The available evidence points to the kidneys as the locus of this regulatory function of the cortical hormone.

In a previous communication (2) we also presented evidence of the interrelationship between the suprarenals and the kidneys by showing that diuresis occurs after suprarenalectomy, and that the nitrogen-concentrating power of the kidneys is impaired. That diuresis is a primary factor, and not due to an increased water intake is shown by the fact that the water intake after suprarenalectomy remained exactly the same as before operation, 21 cc. per day, while the urinary output increased from 1.8 cc. daily during the control period to 6.3 cc. during the first 3 weeks after suprarenalectomy. Swingle and coworkers (7) have shown that suprarenalectomized dogs also excrete a greater volume of water in relation to the total fluid intake than they do during the control period. The enormous increase in urine volume during NaCl treatment,

TABLE I. WATER, CHLORIDE, SODIUM AND POTASSIUM METABOLISM IN RATS BEFORE AND AFTER SUPRARENALECTOMY. DAILY AVERAGES BY PERIODS.

Date 1936	Urine	Fluid Intake	Chloride					Sodium			Potassium			pH
			Urine	Feces	Total Excret.	Intake	Retention	Urinary Excret.	Intake	Retention	Urinary Excret.	Intake	Retention	
	cc.	cc. H_2O	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	
May 19–25	2.7	21	62.2	18.7	80.9	162.1	81.2	29.8	82.4	52.6	20.9	66.1	45.2	7.4
May 26–June 1	2.1	22	75.1	21.7	96.8	166.6	69.8	34.8	84.5	49.7	23.4	67.8	44.4	6.8
June 2–8	2.3	20	68.7	23.8	92.5	179.2	86.7	32.5	90.9	58.4	22.4	73.0	50.6	6.8
June 9–15	2.0	21	73.3	24.4	97.7	144.2	46.5	33.8	73.1	39.3	25.9	58.7	32.8	74.
May 19–June 15	2.3	21	70.1	21.5	91.6	163.0	71.4	32.8	82.8	50.0	23.2	66.5	43.3	7.1
*June 19–25	7.8	21	58.1	14.6	72.7	109.2	36.5	26.8	55.4	28.6	22.9	44.5	21.6	8.6
June 26–July 2	4.8	22	67.1	14.0	81.1	120.4	39.3	29.1	61.1	32.0	24.0	49.0	25.0	8.6
July 3–9	4.2	20	58.9	17.0	75.9	149.8	73.9	27.4	76.0	48.6	21.8	61.0	39.2	8.6
June 19–July 9	5.7	21	60.4	15.4	75.8	127.5	51.7	27.5	64.7	37.2	23.8	51.9	28.1	8.6
		0.5% NaCl												
July 10–16	26.0	72	179.6	28.1	207.7	214.0	16.3	89.2	121.5	32.3	29.1	54.2	25.1	8.6
July 17–23	28.0	60	221.2	26.5	247.7	268.2	20.5	111.9	154.7	42.8	30.6	61.6	31.0	8.6
July 24–30	34.0	71	198.4	35.0	233.4	259.8	26.4	93.8	150.4	56.6	34.3	58.1	23.8	8.6
July 10–30	29.0	68	199.8	29.9	229.7	247.4	17.7	98.4	142.3	43.9	31.4	58.0	26.6	8.6

* Suprarenalectomized on June 16th.

20.8 cc. per day, goes hand in hand with a greatly increased fluid intake, 50 cc. per day. It is well known that suprarenalectomized rats have a greater need and desire for salt than normal animals (8). Since in our experiments the salt was offered in the drinking water, the rats could not satisfy their salt hunger without greatly increasing their fluid intake at the same time. Heller and Haddad (9) state that in their experiments on the effect of saline and alkaline water on normal rats, sodium chloride in the drinking water seems to stimulate food consumption. We also found that the food intake, which had decreased 24 per cent in the period after suprarenalectomy, returned nearly to normal during the last period, when the rats drank 0.5 per cent NaCl solution. It seems, however, more likely that this was due to the relief of suprarenal insufficiency by NaCl treatment, than to the added salt intake as such. The above mentioned authors conclude from their experiments on normal rats that increase in chloride intake increases chloride storage. Increasing chloride intake 73 per cent over that in the control period when 40 per cent of the intake was retained, did not increase chloride storage in suprarenalectomized animals but decreased it to only 16 per cent of the intake.

This decrease was probably due to the diuresis, which washed out the chlorides —the urinary Cl excretion rising 200 per cent. Suprarenalectomy as such does not seem to injure the mechanism for chloride storage, since the retention in percentage of intake stays the same in the period after operation as in the control period. Heller and Haddad show that in normal rats the percentage of fecal excretion rises with the chloride intake. In our experiments on suprarenalectomized animals this change in the path of excretion proceeded in the opposite direction; the fecal excretion in percentage of total excretion dropped from 24 per cent during the control period to 17 per cent after operation

TABLE 2. CALCIUM, MAGNESIUM AND PHOSPHORUS METABOLISM IN RATS BEFORE AND AFTER SUPRARENALECTOMY. DAILY AVERAGE BY WEEKS.

Date 1936	Calcium				Magnesium				Phosphorus			
	Excretion		Intake	Retention	Excretion		Intake	Retention	Excretion		Intake	Retention
	Urine	Feces			Urine	Feces			Urine	Feces		
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
5/19–25	0.3	140.7	169.9	28.9	0.1	8.0	9.3	1.2	1.8	72.5	83.5	9.2
5/26–6/1	0.4	128.4	174.2	45.4	0.1	7.4	9.5	2.0	1.7	73.0	85.7	11.0
6/2–8	0.3	132.8	187.5	54.4	0.1	7.6	10.2	2.5	1.8	73.2	92.2	17.2
6/9–15	0.2	119.8	150.9	30.9	0.1	6.7	8.2	1.4	2.2	62.6	74.2	9.4
5/19–6/15	0.3	130.0	170.6	40.3	0.1	7.4	9.4	1.9	1.9	70.2	83.9	11.8
*6/19–25	0.4	92.8	114.2	21.0	0.1	5.6	6.2	0.5	3.0	46.3	56.2	6.9
6/26–7/2	0.3	101.5	126.0	24.2	0.1	6.0	6.9	0.8	3.5	54.0	61.9	4.4
7/3–9	0.4	105.4	156.8	51.0	0.1	5.2	8.6	3.3	3.8	61.6	77.0	11.6
6/19–7/9	0.4	99.6	133.4	33.4	0.1	5.6	7.3	1.6	3.4	54.0	67.2	9.8
7/10–16	0.7	93.1	139.2	45.4	0.1	6.1	7.6	1.4	4.3	54.7	66.5	7.5
7/17–23	0.5	119.0	158.2	38.7	0.1	7.3	8.6	1.2	5.5	59.9	77.8	12.4
7/24–30	0.4	125.5	149.4	23.5	0.1	6.7	8.2	1.4	4.0	60.2	73.4	9.2
7/10–30	0.5	111.9	148.9	36.5	0.1	6.7	8.2	1.4	4.6	58.2	72.5	9.7

* Suprarenalectomized on June 16th.

and to 9 per cent during the period of NaCl treatment. This drop in fecal excretion tended to counteract the increase in urinary excretion—from 43 per cent to 50 per cent of the intake—in the first period after suprarenalectomy, so that the same percentage of the intake was retained as before operation.

After suprarenalectomy the potassium concentration of the blood rises in dogs and cats, according to Hastings and Compère (10), Urechia, Benetato and Retezeanu (11), Truszkowski and Zwemer (12), and Loeb (13), who, however, found no correlation of this change with changes in potassium balance. In his experiments, while sodium excretion rose markedly, the urinary potassium excretion remained unchanged. We found that in rats, though there was little change in the absolute value of the potassium excreted in the urine, only 54 per cent of the intake was retained during the first 3 weeks after operation, as against 62 per cent during the control period. During the period of NaCl treatment retention dropped to 43 per cent of the intake, most likely due to diuresis.

It has been shown that there is a decrease in the sodium content of the blood serum of patients suffering from Addison's disease (14), as well as of suprarenalectomized cats and rabbits (15). Harrop and Weinstein (16) report a slight drop in the total base of the serum of suprarenalectomized dogs. Loeb (13) states that in suprarenal insufficiency in dogs there is a considerable loss of sodium in the urine. This loss of sodium from the body is an important factor in the development of the clinical picture of suprarenal insufficiency in the dog. Due to its microscopic accessory cortical tissue the rat develops a state of chronic suprarenal insufficiency and probably, therefore, shows no negative sodium balance. Nevertheless, the increase in sodium excretion, though of a lesser order of magnitude, proceeds in the same direction. Retention fell from 59 per cent of the intake before to 53 per cent after operation. During the period of NaCl treatment, in spite of the fact that the intake was more than trebled, only 34 per cent of the intake was retained, due to diuresis. In Loeb's experiments (13) not only the total amount of sodium in the urine but also its concentration was markedly increased, in spite of an augmented urine volume. In rats there was no such increase in concentration, but rather a decrease, from 1828 mg. per 100 cc. of urine to 444 mg. after suprarenalectomy, and 487 mg. during the period of NaCl treatment. We found here the same impairment of the concentrating power of the kidneys as we had previously noted in regard to nitrogen (2).

Suprarenalectomy does not seem to have any important influence on calcium metabolism. The blood calcium level remains unchanged in cats (17). Pugsley and Collip state that no significant difference in the calcium excretion in the urine is found after suprarenalectomy. Nevertheless, they conclude on the basis of their studies on the effect of parathormone on suprarenalectomized rats that calcium is more readily mobilized from the bones after suprarenalectomy. We also found only a slight rise in urinary calcium excretion after operation. The rise of 100 to 200 per cent during the period of NaCl treatment was doubtless due to diuresis.[1]

The excretion of magnesium closely followed that of the calcium ion. Urinary magnesium excretion did not differ from that during the control period either after suprarenalectomy or during the period of NaCl treatment, contrary to the rise in urinary calcium excretion. But the absolute values were so small, the main way of excretion being the gut in both cases, that retention was not influenced; about 22 per cent of the intake of calcium as well as of magnesium was retained, the same as before operation.

Harrop (19), working on dogs, suggests that since the water available as a vehicle for the urinary excretion of dissolved substances, in the absence of the suprarenal cortical hormone, is particularly claimed by sodium and chloride, the excretion of other materials probably is impeded. In rats we have found the opposite to be the case. Urinary nitrogen and sulfur excretion increased (2), and there was also a marked rise in urinary phosphorus excretion, 80 per cent during the first 3 weeks after operation, and 130 per cent during the period of NaCl treatment. This rise, though definite, would not

[1] This paper came to our attention only after the completion of our experiments.

materially influence retention, since the absolute value of the urinary phosphorus excretion remained small. The loss of phosphorus by way of the gut also increased, so that retention dropped somewhat, from 15 per cent of the intake during the control period to 11 per cent after operation, and to 8 per cent during the period of NaCl treatment.

SUMMARY

The water, chloride, potassium, sodium, calcium, magnesium, and phosphorus metabolism of suprarenalectomized rats untreated, or maintained on 0.5 per cent NaCl solution, was studied. Water intake remained constant after suprarenalectomy, but rose during NaCl treatment. The urine volume increased. Urinary chloride excretion increased, but the mechanism for chloride storage was not injured by suprarenalectomy, since a drop in fecal excretion counteracted this rise, so that retention in percentage of intake remained constant. During the period of NaCl treatment, retention decreased considerably, due to diuresis. The absolute value of the potassium excreted in the urine showed little change, but there was a drop in the percentage of potassium retained. Urinary sodium excretion increased, but the concentration of sodium per 100 cc. of urine fell markedly. Calcium and magnesium retention remained unchanged. Urinary as well as fecal phosphorus excretion increased slightly. The significance of these changes has been discussed.

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OBSERVATIONS ON CARBOHYDRATE METABOLISM FOLLOWING IRRADIATION OF THE PITUITARY GLAND

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Various investigators have presented evidence to show that the hypophysis plays an important rôle in carbohydrate metabolism. Houssay (1) and his coworkers were the first to show that extracts of the anterior lobe of the hypophysis, when injected into normal dogs, produce hyperglycemia, glycosuria and ketonuria (2). Furthermore, these extracts, when given in high doses, inhibit dextrose utilization and a diabetic condition is produced. The ablation of the pituitary leads to hypersensitivity to insulin, hypoglycemia, convulsions during fasting, and an increase of dextrose tolerance (3, 4). All these findings have received adequate confirmation. In depancreatized dogs the removal of the hypophysis restores carbohydrate metabolism to an approximately normal condition but on the other hand renders the animal hypersensitive to insulin (5, 6). The injection of the anterior lobe extracts to the depancreatized-hypophysectomized animal causes a reappearance of the symptoms of diabetes. This subject has been reviewed in full by Houssay (7).

Since the removal of the hypophysis decreases the symptoms of experimental diabetes, it has been advocated that destructive doses of roentgen ray to the hypophysis might produce similar results in clinical diabetes. Studies in carbohydrate metabolism, usually by simple dextrose tolerance curves in patients with diabetes mellitus before and after irradiation, have been made in the past by Selle, Westra and Johnson (8, 9), and Hutton (10). These investigators found little or no changes in dextrose tolerance after irradiation and there was no increased sensitivity to insulin. However, Allen and Lisser (11) note that roentgen-ray therapy to the hypophysis in acromegaly may favorably influence dextrose tolerance. An analysis of their data shows considerable variation in the types of curves which they have obtained. Furthermore, improvement in the dextrose tolerance curve may occur gradually during a long dietary regime on which their patients were placed, and the diabetes of acromegaly differs considerably from the ordinary diabetes mellitus by reason of its fluctuating character. In short, up to this date there have been no clear-cut changes demonstrated in carbohydrate metabolism following irradiation of the pituitary body in experimental animals or human subjects with diabetes mellitus or evidences of pituitary tumor or dyscrasia.

We were afforded the opportunity to carry out certain studies of carbohydrate metabolism before and after roentgen-ray therapy, on a group of

patients at the Peter Bent Brigham Hospital who were to be subjected to considerable irradiation of the pituitary body for the treatment of the menopausal syndrome. These cases offered an opportunity to study the effect on carbohydrate metabolism of irradiation to an apparently normal pituitary, and were carried out before irradiation and again 4 to 40 days after irradiation. In addition, the dextrose tolerance curves were determined after basal conditions had been well established. Ten cases in apparently good health, except for the complaints consistent with the menopausal syndrome, were subjected to roentgen irradiation to the pituitary gland. The ages of these patients varied from

TABLE 1. SUGAR TOLERANCE FOLLOWING THE ORAL ADMINISTRATION OF 1 GM. OF DEXTROSE PER KG. BODY WEIGHT, BEFORE AND AFTER IRRADIATION TO THE PITUITARY BODY. BLOOD SUGAR VALUES IN MG. PER CENT.

	Irradiation	Fasting	20 Min.	40 Min.	60 Min.	80 Min.	100 Min.	120 Min.
S. A.	Before	71	98	108	146	145	120	105
	5 Days after	67	110	128	150	133	115	98
M. S.	Before	95	118	130	142	150	126	108
	9 Days after	97	122	138	146	136	115	102
E. R.	Before	77.5	130	149	146	132	114	106
	6 Days after	82	126	140	150	128	116	110
C. B.	Before	86	118	134	152	130	121	112
	7 Days after	79	100	120	146	128	118	104
A. F.	Before	69	98	120	132	121	112	100
	5 Days after	74	108	119	126	113	110	106
A. S.	Before	74	111	126	137	118	113	107
	7 Days after	73	101	101	122	122	114	108
E. A.	Before	73	120	131	170	152	133	118
	6 Days after	79	129	140	164	148	128	116
R. P.	Before	62	113	117	118	117	110	102
	40 Days after	71	116	120	128	115	108	102
P. W.	Before	60	75	125	128	121	100	90
	4 Days after	64	80	118	120	115	102	94
R. G.	Before	91.3	156	145	143	140	138	118
	4 Days after	70	131	142	156	131	120	110

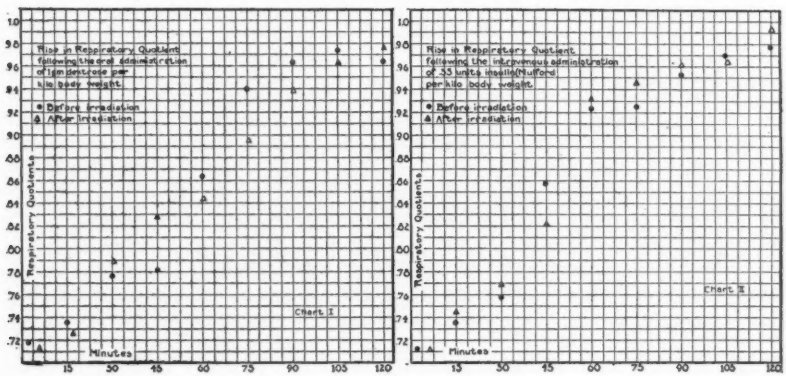
29 to 50 years, with an average age of 42 years. The patients received a total of 1400 to 1600 R skin doses over a period of 4 days. The irradiation was sufficient to cause the loss of the patient's hair over the temple region. Thirty-five per cent had a definite and sustained decrease in the number of their flashes associated with menopausal syndrome. No ill effects, except an occasional headache, were noted in any of the patients following the treatment.

Dextrose tolerance tests were carried out after basal conditions had been well established. In some cases the patients were brought into the hospital for a period of time in order to adequately control these conditions. One patient was placed in bed at complete rest and blood sugar and respiratory metabolism determined. She was then given 1 gm. of dextrose per kg. of body weight orally, and blood samples were withdrawn for analysis every 20 minutes for a

TABLE 2.—BLOOD SUGAR VALUES FOLLOWING INTRAVENOUS ADMINISTRATION OF 0.35 U. INSULIN (MULFORD) PER KG. BODY WEIGHT.

	Irradiation	Fasting	20 Min.	40 Min.	60 Min.	80 Min.	100 Min.	120 Min.
S. A.	Before	96	80	72	64	62	56	58
	After	86	76	70	66	60	54	56
M. S.	Before	84	72	64	58	54	58	60
	After	88	76	66	52	54	61	62
E. R.	Before	72	64	58	54	53	56	56
	After	79	67	60	56	54	56	60
C. B.	Before	94	85	42	42	48	52	52
	After	84	72	56	48	52	54	56
A. F.	Before	71	68	50	42	46	50	52
	After	76	65	55	52	50	50	54
A. S.	Before	94	86	76	65	56	56	58
	After	98	79	72	60	54	56	58
E. A.	Before	76	64	60	56	54	54	58
	After	84	71	63	56	51	52	54
R. P.	Before	93	86	72	66	60	59	59
	After	95	84	80	76	62	60	62
P. W.	Before	99	96	91	80	64	60	60
	After	86	84	82	70	60	62	62
R. G.	Before	84	75	64	60	54	56	56
	After	90	80	65	62	58	59	58

period of 2 hours. Simultaneous samples of expired air were collected from an aliquot apparatus attached to a Tissot spirometer. Practise experiments were occasionally carried out until the ventilation rates were constant. The gas analyses were made by the use of a Haldane-Boothby apparatus and at the onset of each experiment an outside air determination was done. Blood-sugar determinations were carried out by the Folin micro-sugar method, using venous blood. The blood samples were drawn from a large vein in the antecubital fossa without stasis produced by a tourniquet. The same procedure was carried out after giving intravenously 0.35 U. of insulin per kg. of body weight. As the


Rise in Respiratory Quotient following the oral administration of 1 gm dextrose per kilo body weight.
● Before irradiation
▲ After irradiation
Respiratory Quotients
Minutes
Chart I
Rise in Respiratory Quotient following the intravenous administration of .35 units insulin (Mulford) per kilo body weight.
● Before irradiation
▲ After irradiation
Respiratory Quotients
Minutes
Chart II

results were fairly consistent we shall present the data as a group of sugar tolerance tests in table 1, and the isolated experiment with respiratory metabolism in table 2. It will be seen that there was no great disturbance in sugar tolerance either before or after irradiation.

In Chart I it will be noted that the rise in respiratory quotient following the administration of dextrose was well within normal limits. The ventilation rates were remarkably steady and there was no abnormal deviation of the oxygen consumption after the administration of dextrose. As destruction of the pituitary body results in hypersensitivity to insulin, the same procedures were carried out with insulin. Such changes are often more striking in the experimental animal than changes in sugar tolerance, but it will be seen from table 2 and Chart II that there was no increased sensitivity to intravenous insulin.

DISCUSSION

Massive irradiation to the pituitary body cannot be directed entirely to the pituitary itself, but must act as well on nervous centers within the proximity of this gland. If changes in carbohydrate metabolism did take place it would be extremely difficult to evaluate the findings or to ascribe them to changes in this one gland alone, for one would have to consider the effect of irradiation on the vegetative centers in the hypothalamus as well. It would appear from the data presented that to employ roentgen ray in the treatment of diabetes or other possible disturbances of the carbohydrate metabolism on the pituitary body would be attended with considerable uncertainty. Selle, Westra and Johnson (8, 9) noted that in 5 of 7 diabetic dogs the fasting blood sugar was not reduced after irradiation of the hypophysis by high dosage and in some cases the blood sugar was considerably higher after irradiation. Furthermore, the response to ingested glucose was always typical of severe diabetes.

SUMMARY

The treatment of the menopausal syndrome by 1200 to 1600 R of irradiation to the pituitary gland caused no changes in carbohydrate metabolism.

The authors take this opportunity to thank Dr. Merrill C. Sosman for his kind assistance with this publication.

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THE EFFECT OF FEMALE SEX HORMONES UPON THE OXYGEN CONSUMPTION RATE OF NORMAL RATS, AND UPON THE TOLERANCE TO DESICCATED THYROID

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In a previous report (1) it was shown that pregnant rats tolerate doses of desiccated thyroid which are definitely toxic when fed to non-pregnant animals. Bodansky and Duff (2) made a similar observation. The question immediately arises whether the hormones which are present in high concentration during pregnancy play a role in this increased resistance. The present study is an attempt to answer this question at least in part by determining whether the administration of these hormones increases the tolerance of otherwise normal rats to desiccated thyroid. Also, in view of the varied reports by other workers in this field, the effect of these substances themselves upon oxygen consumption was studied.

That the hormones of the thyroid gland and of the gonads have an effect of mutual antagonism under certain conditions has been established. Kippen and Loeb (3) have found that extirpation of the gonads in sexually-mature guinea pigs leads to an increase in the proliferative activity of the thyroid during the first week following operation, and also found other evidences of stimulation which might, theoretically, be attributed to withdrawal of gonadal antagonism. Bialet-Laprida (4) has shown that the administration of 2 to 5 R.U. of estrone daily before maturity caused histologic evidence of thyroid hypofunction after maturity was reached. They conclude that estrone inhibits the thyroid either by direct action or indirectly through the anterior pituitary. Lundberg (5), Heyl, deJongh and Kooy (6), and Benazzi (7) noted other evidences of thyroid inhibition by estrone. Reiss and Pereny (8) found that the thyroid hormone was antagonistic to estrone in both the castrate and normal animal. Englehart (9) noted an antagonistic effect of thyroxin on the corpus luteum. Starr and Patton (10, 11) produced remissions in hyperthyroidism in a series of women of child-bearing age by the administration of pregnancy urine extract. Sherwood, Savage and Hall (12) noted a drop in metabolism of 13 to 42 per cent in rabbits following the injection of 10 to 800 R.U. of amniotin. They state that after a rabbit had been given a very large ('nearly lethal') dose of thyroid and a high B.M.R. had been obtained, amniotin was administered and the basal rate dropped promptly to —8 per cent. In a later article, Sherwood and Bowers (13) reported that after experimental hyperthyroidism had been produced, the administration of amniotin caused the B.M.R. to return to the normal level in one-half the time normally required. VanHorn (14) showed that in castrated rats in a hyperthyroid condition approximately 3 times the normal amount of estrone was necessary for the production of artificial estrus.

EXPERIMENTAL PROCEDURE

The animals used in this study were female albino rats of approximately the same age (6 months) and weight (180 gm.) and from the same colony. All of the rats received desiccated thyroid except during the early control period. A dose of 100 mg. was added to the food ration together with 2 to 3 cc. of water to make a paste. Oxygen consumption determinations formed the means by which the effect of thyroid feeding was measured. The apparatus used for this purpose was a modification of that recommended by Tainter and Rytand (15).

The normal rate of oxygen consumption of each animal was determined by taking several trial readings before treatment was begun. Then, the hormones used, except in the aqueous estrone series, were administered for 7 days before thyroid feeding was begun in an endeavor to note their effect, if any, upon the normal animal. Thyroid feeding was then started (the hormone administration being continued as during the preceding week) and continued for 14 days.

The rats were fed and/or injected at about 4 P.M. daily, and oxygen consumption measured at about 12 noon 3 times weekly. They were not fasted prior to the measurement of the metabolic rates, since comparative rates only were desired. This routine was adhered to in order to rule out, as nearly as possible, diurnal changes in weight and activity.

The following preparations were tested for their effect upon oxygen consumption and for their efficacy in inhibiting the metabolic effects of orally administered thyroid: *estrone,* in the form of aqueous theelin (Parke, Davis and Company), theelin in oil (P. D. & Co.), and amniotin (Squibb); *estriol,* in the form of theelol (P. D. & Co.); *emmenin,*[1] the estrogenic factor of the placenta (Collip; Ayerst, McKenna and Harrison, agent); the *anterior-pituitary-like substance of pregnancy urine* in the form of antuitrin-S (P. D. & Co.); and *progesterone,* in the form of lipolutin (P. D. & Co.).

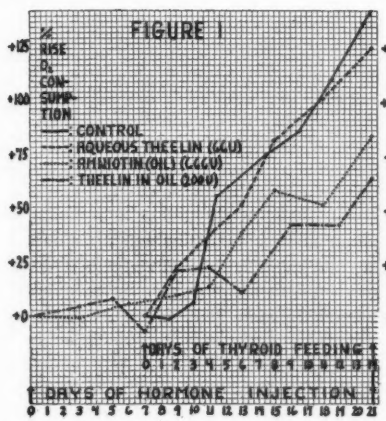
The oxygen consumption rates were analyzed statistically in order to determine whether the changes shown were significant. In all of the results which follow, the changes in oxygen consumption resulting from the administration of the hormones alone were found to be not significant; the rise in oxygen consumption following the administration of desiccated thyroid was found, in each series, to be significant.

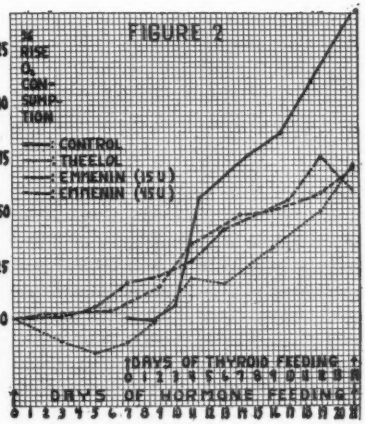
A. The *control* group consisted of 20 animals, each of which received 100 mg. of desiccated thyroid daily. By the end of 14 days of thyroid feeding the average percentage rise in oxygen consumption was 134 ± 3.2.

B. The animals which received *estrone* were divided into 3 groups. The first group consisted of 10 rats, and received subcutaneously 67 I.U. of aqueous theelin plus 100 mg. of desiccated thyroid in the food, daily. At the end of 14 days of such treatment the percentage rise in oxygen consumption was 123 ± 3.2. The animals in the second group (of which there were 6) received 200 I.U. of theelin in oil subcutaneously, daily. After 7 days of administra-

[1] The emmenin which was used in this study was generously supplied by Dr. W. J. McKenna of Ayerst, McKenna & Harrison (United States) Limited.

tion of the hormone alone, there was found to be no significant change in oxygen consumption. At the end of 14 days of thyroid feeding plus theelin, the average percentage rise in oxygen consumption was 63.4 ± 5.0. The animals in the third group, which consisted of 10 rats, received 666 I.U. of amniotin daily. The change in oxygen consumption as a result of this treatment alone was not significant. After desiccated thyroid had been administered daily for 14 days, the average percentage rise in oxygen consumption was 82.6 ± 5.7.





C. Emmenin was administered to 16 rats by mixing it with the daily food ration. Of these, 10 received 15 'day units' daily; after 7 days of such treatment there was no significant change in oxygen consumption. After 14 days of thyroid feeding plus emmenin, the average percentage rise was 69.5 ± 3.0. The remaining 6 rats received 45 'day units' daily. After 7 days there was no significant change; after 14 days of thyroid feeding plus emmenin, the average percentage rise was 51.0 ± 4.1.

D. Estriol was administered to 6 rats by mixing it with the daily food ration. Each animal received 200 I.U. daily. After 7 days of theelol alone there was no significant change; after 14 days of thyroid feeding plus theelol the average percentage rise was 70.6 ± 5.4.

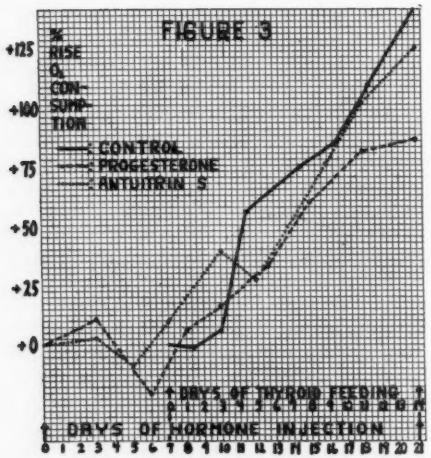
E. Progesterone was administered to 6 rats, each of which received 0.2 Rb.U. subcutaneously, daily. After 7 days of such treatment there was no significant change in oxygen consumption; after 14 days of thyroid feeding plus lipolutin the average percentage rise was 86.1 ± 12.1.

F. A.P.L. factor or pregnancy urine was administered to 6 rats subcutaneously in dosage of 20 R.U. daily. After 7 days of antuitrin-S alone there was no significant change in oxygen consumption. After 14 days of thyroid feeding plus antuitrin-S, the average percentage rise was 105.0 ± 8.2.

DISCUSSION

A. Effect of female sex hormones upon oxygen consumption rates of

normal animals. It has been found in this study that the hormones tested have no significant effect upon the oxygen consumption rates of normal rats. When the results are not analyzed statistically, however, certain percentage changes appear which might be interpreted as due directly to the administration of these substances. When the probable errors are taken into account, however, these figures are shown to differ in no significant manner from those taken during the control period. This failure to employ the statistical method may readily explain the great variety of effects obtained by other workers who have made tests similar to those outlined here. For example, Sherwood, Savage and Hall, and later Sherwood and Bowers, report a decrease in basal metabolism as a result of estrone injection. However, since no mention is made of the mathematical significance of these results, an element of uncertainty remains as to



whether the changes which they describe are true ones or are only apparent. McClendon and Burr (16) reported as increase in the basal metabolism of a woman after the administration of estrone. Kunde, D'Amour, Carlson and Gustavson (17) conclude from their work upon the dog that estrone does not affect the basal metabolic rate. King and Patterson (18) administered the hormone to women and found no change in basal metabolism. Since it is hardly conceivable that there is a species specificity to estrone which in some animals is manifest by an increase in metabolism, in others by a decrease in metabolism, and in a third group by no effect at all, one is forced to the conclusion that in certain instances either the analyses themselves were inaccurate, or that the results as reported are not significant.

Our results are in accord with those of Kunde, D'Amour, Carlson and Gustavson and of King and Patterson, namely, that this hormone has no significant effect upon the oxygen consumption.

The other hormone preparations tested were also found to be without effect in this regard. It has been shown by other workers that the hormones

of the thyroid gland and of the gonads have, under certain conditions, an effect of mutual antagonism. It seems not unreasonable to suppose that in the intact animal the inhibitory effect (if any) of the female sex hormones upon the thyroid hormone is compensated for by the increased 'sexual' activity, maintaining thereby the metabolic rate at its normal level.

B. Influence of female sex hormones upon the metabolic effect of orally administered thyroid. It is shown that the metabolic effect of orally administered thyroid, as determined by oxygen consumption rate, is definitely diminished by treatment with these hormones. When compared statistically with the controls, the results are found to be significant. These results, though statistically different from one another in many instances, may be grouped conveniently as follows.

1. Estrone Group. A significant inhibition of the oxygen consumption rate was observed with each of the 3 dosages employed. The very slight inhibition resulting from treatment with the aqueous preparation may be due to one or both of two factors. First, the lesser dosage employed may be responsible, or, second, the hormone in the aqueous solution may have been too rapidly absorbed and excreted to give a measurable, continuous inhibition of oxygen consumption. The difference in effect produced by the two oil solutions is barely significant, when the figures are analyzed statistically.

2. Estriol and Emmenin Group. In view of the fact that the physiologically-active fraction of emmenin is probably the same as that of theelol (19), their consideration together seems warranted. Although the higher dosage of emmenin seems to give a greater inhibition at the end of 14 days of thyroid feeding, a comparison of the graphs shows no essential difference between the effects of the two dosages. Since a large absolute inhibition was obtained with the smaller dose of emmenin, and since increasing the dose by 3 times had but little effect in increasing this inhibition, one is justified in concluding that there is a physiologic limit beyond which experimental hyperthyroidism cannot be restrained. A similar inference might be drawn when the statistically similar results of the two estrone in oil groups are compared. The theelol curve parallels the emmenin curves closely. A comparison of the dosage-effect ratios of emmenin and theelol, however, is impossible because of the different methods of standardization and definition of the units.

3. Progesterone. The inhibition obtained with progesterone, though significant, was less than that of the theelol-emmenin group.

4. Anterior Pituitary-like Substance. A small but significant inhibition was obtained with antuitrin-S. This inhibition, with the exception of the low dosage estrone group, was the least of any of the hormones used. Again, the amount administered may have been too small for a full effect. The interpretation is difficult because of the varied physiologic effects of antuitrin-S. With stimulation of the ovaries an increased amount of estrone and, in the rat at least, of progesterone is present in the organism. The inhibition therefore may be due to the antuitrin-S per se, to the estrone, to the progesterone, or to the combined action of one or more of these factors.

As previously indicated, this work was undertaken in order to determine,

if possible, whether the high concentration of estrogenic and gonadotropic hormones during pregnancy was the factor responsible for the inhibition of the metabolic effect of administered thyroid during this period. It was found that each of the hormones tested was able to inhibit this effect, but not to as great a degree as that found during pregnancy. It was found further that not only one, but all of these substances possessed this restraining potentiality if administered in sufficient dosage.

On the basis of the present study a specific answer concerning the mechanism of the action of the active principles used is impossible. Certain investigators have reported that the thyroid is specifically inhibited by the female sex hormones. We have found that the oxygen consumption rate does not change significantly after the administration of the female sex hormones, and that the oxygen consumption rate increases more in animals which receive desiccated thyroid alone than in those which receive female sex hormones plus a similar amount of thyroid. These observations indicate that the mechanism involved in yielding the results in our experiments is not a simple direct inhibition of the thyroid by the administered hormones. It would appear on first consideration that the sex hormones are more effective in antagonizing the effect of excessive amounts of thyroxin than in inhibiting the normal secretory activity of the gland. Such a conclusion is not warranted, however, because other possibilities have not been ruled out, namely: *a)*, increased sexual and bodily activity caused by the hormones may increase oxygen consumption and obscure any inhibition of the thyroid possibly resulting from the hormones; *b)*, the thyroid may compensate adequately in the presence of an inhibitory effect exerted by the hormones; *c)*, we do not know how the thyrotropic and gonadotropic activity of the anterior lobe may be affecting the activity of the thyroid and metabolism of the body cells. The question is complicated by these and other factors unnecessary to mention, and cannot be answered until all the possibilities are properly controlled.

In explanation of the increased tolerance of pregnant rats to desiccated thyroid as found previously (1), one is justified in concluding only that the female sex hormones which are present in high concentration at this time may constitute one of the factors involved.

SUMMARY

Large doses of estrone, estriol, emmenin, progesterone and the A.P.L. factor of pregnancy urine, when administered to female albino rats, had no significant effects upon the rate of oxygen consumption. After a week of such treatment, 100 mg. of desiccated thyroid per rat was fed daily for 14 days. The increase in the oxygen-consumption rate in all of these doubly-treated groups was significantly less than that in control rats given thyroid alone. There is a physiologic limit beyond which the metabolic effect of orally-administered thyroid cannot be inhibited by the presence of these hormones in excess.

Wherever applicable, statistical methods should be employed in the analysis of such results. This procedure obviates any question as to the significance of reported figures and greatly simplifies their evaluation.

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EXPERIMENTAL ACUTE HYPERPARATHYROIDISM. 1. CHEMICAL STUDIES

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The purpose of the present investigation was to determine: *1)* the influence of different plasma protein concentrations upon the diffusibility of serum calcium and the degree of hypercalcemia produced by the administration of massive doses of parathyroid extract; *2)* the effect of the hypercalcemia upon the diffusibility of the inorganic P of the serum; *3)* the changes, if any, in the ester P of the corpuscles and the phosphatase activity of the plasma during states of severe acute hyperparathyroidism.

EXPERIMENTAL PROCEDURE

Parathyroid extract (Parke, Davis and Co.)[1] was injected intramuscularly in 6 adult dogs (9-18 kg.), in doses of 100 to 300 U. each, at 9 A.M., 12 M., 3 and 5 P.M. daily for 3 days, the total amount of extract administered ranging from 2700 to 3500 U. Food was withheld during the experimental period but water was given as desired. Two animals were rendered anemic by preliminary bleeding and, in one case, an attempt was made to reduce the plasma protein concentration by plasmapheresis and protein restriction, prior to the injection of the extract. The following determinations were made before and once or twice daily during the experimental period, blood being obtained from the heart or femoral artery (about 30 cc.): hematocrit, blood NPN (1), total plasma protein and albumin (2), serum calcium (3) serum inorganic phosphorus (4), diffusible calcium and inorganic phosphorus (5), serum phosphatase activity (6) and corpuscular ester P (7). One animal died at the end of the third day; the remainder were killed by bleeding under light ether anesthesia and were autopsied after 72 hours of repeated parathyroid administration.

RESULTS

All animals presented the characteristic clinical manifestations of acute hyperparathyroidism terminating in renal failure, as described by Collip, with anorexia, vomiting, hematemesis, bloody diarrhea and, toward the end of the experimental period, marked respiratory distress. The blood NPN rose from control levels of 23 to 36 mg. per 100 cc. to 77 to 86 mg., values above normal being obtained first at the end of 48 hours in each instance. The plasma CO_2 combining power fell to terminal levels of 29 to 38.5 volumes per cent.

[1] The parathyroid extract was furnished through the generosity of Dr. E. A. Sharp, of Parke, Davis & Co.

Hematocrit. The control hematocrit values ranged from 26 to 49. At the end of the experimental period these values had fallen in 3 (8.1, 15 and 20 per cent below original level) and risen in 3 (2.5, 3.9 and 6.6 per cent above original level) instances. In those cases in which a drop in hematocrit occurred it reached its maximum at the end of 24 hours, little change being noted subsequently.

Serum Proteins. The total serum protein concentration increased during the experimental period in every case. The control, maximum and terminal values were as follows: *1)*, 4.07, 4.68 (24 hr.), 4.55 gm. per 100 cc.);

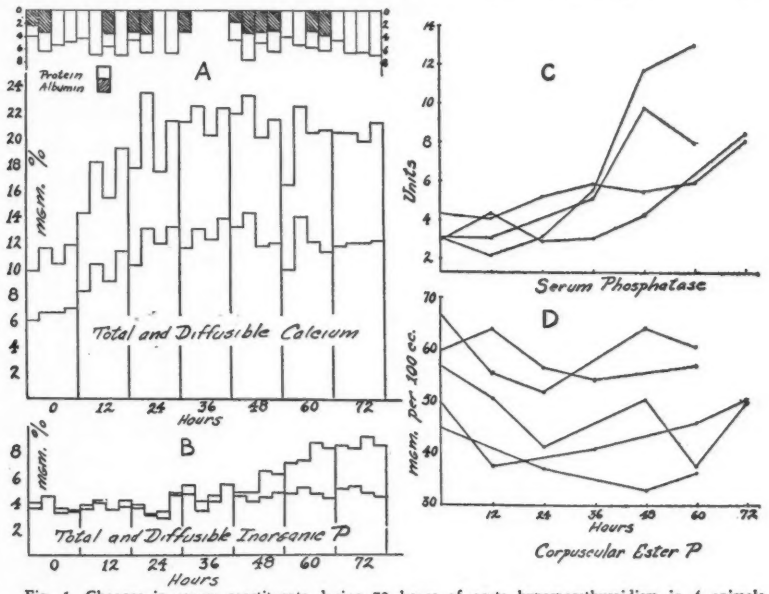

Fig. 1. Changes in serum constituents during 72 hours of acute hyperparathyroidism in 4 animals. *A*, Total protein, albumin, and total and diffusible inorganic Ca. *B*, Total and diffusible inorganic P. *C*, Serum phosphatase. *D*, Corpuscular ester phosphorus.

2), 6.27, 7.73 (48 hr.), 6.56; *3)* 5.6, 6.43 (72 hr.); *4)*, 5.14, 7.01 (12 hr.), 6.84; *5)*, 6.18, 6.76 (48 hr.), 6.62; *6)*, 5.28, 6.82 (72 hr.).

The albumin concentration exhibited relatively insignificant variations except in one case in which it fell from 2.67 to 1.98 gm. per 100 cc. The range of albumin values was from 1.98 to 3.92 gm.

Serum Calcium. In 4 cases in which calcium determinations were made at 12 hour intervals the control values ranged from 9.9 to 12 mg. per 100 cc. A sharp rise occurred promptly (fig. 1), a maximum level of 20.3 to 23.6 mgm. being attained usually in 24 to 48 hours and being maintained approximately throughout the experimental period. The degree of elevation of serum calcium bore no demonstrable relation to the serum protein concentration, and was apparently unaffected by variations in the latter within the range (4.09 to 7.73 gm. per cent) of values obtained during the period of maximal hypercalcemia (24-72 hr).

The diffusible calcium increased from control values of 6.06 to 7.1 mg. per cent to a maximum level of 12.2 to 14.3 mg. (fig. 1), the ratio of diffusible to total calcium remaining essentially unchanged throughout (control ratio 57-65 per cent; ratio at height of diffusible fraction 60-62 per cent). The diffusibility of the serum calcium was not appreciably influenced by the concentration of either total protein or albumin within the range of values for the latter factors.

Inorganic P. The serum inorganic-P increased from control values of 3.44 to 4.48 mg. per 100 cc. to 7.18 to 7.62 mg. at the end of the experimental period. The diffusible inorganic-P (control values 3.26-4.5 mg. per cent) remained practically unchanged throughout, the degree of diffusibility, therefore, steadily diminishing in proportion to the increase in inorganic P, from practically 100 per cent in the resting state to 48 to 59 per cent at the termination of the experiment (fig. 1).

Corpuscular Ester-P. (Fig. 2). With the exception of one case, which exhibited a slight primary increase, the corpuscular ester-P diminished steadily from the control level of 45 to 65.2 mg. per 100 cc. to a minimum of 32.6 to 54.5 mg. in 12-48 hours, with a subsequent increase to a terminal level which reached that of the pre-injection state in only one case.

Serum Phosphatase (Fig. 2). There was a gradual increase in serum-phosphatase activity from control levels of 3.06 to 4.18 units to a maximum of 7.18 to 13.15 at 48 to 72 hours.

DISCUSSION

There is considerable evidence that the calcium content of body fluids is in part a function of their protein content and that the influence of the latter in this connection is exercised through the formation of a non-diffusible calcium-protein complex. However, although variations in serum-protein concentration, as in nephrosis and multiple myeloma, have been found to be accompanied by changes in total and non-diffusible calcium, evidence is at hand which indicates that the latter may increase with little or no associated increase in protein. Smith and Sternberger (5) and Benjamin and Hess (8) found that when the serum calcium of normal dogs is increased by intravenous injection of wholly diffusible calcium salts, the relationship between the diffusible and non-diffusible fractions remains essentially normal. Similar results were obtained following the addition of calcium chloride to blood serum in vitro. The physiological increase (100-200 per cent) in the serum calcium concentration of hens during the laying period is due almost entirely to an increase in the non-diffusible fraction, usually with comparatively little associated increase in protein, although Benjamin and Hess obtained protein values during this period about 50 per cent higher than the normal for this species. The observations of Moritz (9), Snell (10), Greenberg and Gunther (11) and Benjamin and Hess (8) and the results of the present study indicate that the administration of parathyroid extract and viosterol results in a practically proportionate increase in both diffusible and non-diffusible calcium. Our observations are not, however, in accord with the statement of Schmidt

and Greenberg (12) that with increases of only 2 to 3 mg. per 100 cc. in blood calcium the rise is reflected almost entirely in the diffusible fraction.

There has been considerable conjecture regarding the physico-chemical condition of the portion of non-diffusible calcium apparently in excess of that bound to protein. Several investigators have suggested that it may be a colloidal form of calcium phosphate, the existence of which, experimentally, as stated by Schmidt and Greenberg (12), "is best demonstrated by a concomitant decrease in the diffusibility of both serum calcium and serum phosphate." This hypothesis is supported by the observation of Grollman (13) and others that the diffusibility of serum phosphate, which is normally practically complete, diminishes when the serum calcium concentration is increased. Furthermore, Greenberg and Tufts (14) have found that when calcium is added to blood serum in vitro, the resulting decrease in diffusibility of inorganic phosphate is paralleled by an increase in non-diffusible calcium. However, this view is not entirely supported by the data reported here. Although a distinct increase in the non-diffusible fraction of serum Ca was present at the end of 12 hours and was marked after 24 hours of parathyroid administration, no significant diminution in phosphate diffusibility could be demonstrated until the end of 36 hours. Furthermore, whereas the diffusibility of the serum phosphate decreased steadily and to a marked degree during the last 36 hours of the experimental period, no significant alteration occurred during that time in either the amount or proportion of non-diffusible calcium. It is difficult to reconcile these findings with the hypothesis that the increase in this factor is due largely or entirely to the formation of a non-diffusible calcium-phosphate compound.

The observations of Smith and Sternberger (5) led them to conclude that the serum protein is normally not saturated with calcium and that when hypercalcemia is induced by the addition of a diffusible Ca salt, in vivo or in vitro, the protein is capable of combining with more than the normal amount. It is of interest in this connection that whereas no consistent quantitative relationship could be demonstrated between the degree of proteinemia and the level of either total or nondiffusible calcium, a definite increase in serum protein concentration occurred in every case during the experimental period. This may perhaps be attributed to the condition of hemoconcentration which accompanies the acute hyperparathyroid state and which was further evidenced by the absence of a progressive fall, and in some cases an actual increase in hematocrit values despite the continued withdrawal of relatively large quantities of blood. The data presented by Benjamin and Hess (8) indicate a similar increase in serum protein concentration in association with the hypercalcemia induced by the administration of calcium gluconate, parathyroid extract or viosterol. In view of these observations it would appear that more careful consideration must be given to the following factors in attempting to interpret the changes in serum calcium which follow the administration of parathyroid extract: *1)*, the effect of hemoconcentration per se upon the level of total and nondiffusible calcium under the existing conditions; *2)*, the increased serum protein concentration; *3)*, the possible state of unsaturation of protein with calcium under normal conditions.

The striking diminution in ester P, despite repeated bleeding, which, according to Ashley and Guest (15), tends to produce an increase, may be of fundamental significance. The subsequent rise in ester P is possibly dependent upon the supervention of renal functional impairment which was found by Byrom and Kay (16), Hoesch (17) and Ashley and Guest (15) to be accompanied by an increase in this factor. The effect of the parathyroid hormone in this connection has received little attention. Sykes, Taylor and Weld (18) reported a slight increase in the total acid-soluble phosphorus of whole blood at the height of the hypercalcemia produced by parathyroid extract. It is impossible to interpret their data accurately because of the lack of information regarding the relative corpuscular volumes at different stages of the experiment. However, if the degree of hemoconcentration in their animals was comparable to that which usually occurs under similar conditions, their findings would appear to indicate a definite decrease in corpuscular ester-P. Stearns and Warweg (19) obtained extremely low ester-P values in a child with hyperparathyroidism which, however, they interpreted as probably due to poor nutrition. This possible interpretation of the findings reported here cannot, of course, be entirely disregarded, but the fact that the drop usually occurred promptly would appear to render such an explanation unlikely.

The decrease in ester P following the administration of parathyroid extract is particularly significant in view of the fact that Guest and Warkany (20) found an increase in rabbits after the administration of large amounts of vitamin D and in view of the marked decrease reported in experimental and clinical rickets by Kay (21), Jacobsen (22), Bakwin, Bodansky and Turner (23) and Stearns and Warweg (19). This observation, in conjunction with the opposite effects of vitamin D and parathyroid extract upon serum phosphatase activity may be regarded as additional evidence that the modes of action of these two agents are not fundamentally identical.

SUMMARY

A state of acute hyperparathyroidism was induced in 6 dogs by the repeated administration of 100 to 300 U. of parathyroid extract 4 times daily for 3 days. All animals presented the characteristic clinical manifestations of acute hyperparathyroidism, with terminal rise in blood NPN and fall in plasma CO_2 capacity. The serum protein concentration increased, due probably to hemoconcentration. There was a practically proportional increase in both diffusible and non-diffusible fractions of serum calcium, which reached their maximum levels after 24 to 36 hours. The inorganic P of the serum rose terminally, but little change occurred in the diffusible fraction throughout the course of the experiment. The ratio of diffusible to total calcium bore no consistent relation to the level of either total protein or albumin within the range of values observed. There was a striking initial diminution in corpuscular ester P, with a subsequent rise, due probably to the supervention of renal functional impairment. The serum phosphatase increased steadily throughout the course of the experiment.

The data presented suggest that: *1)*, the modes of action of the para-

thyroid hormone and vitamin D are not fundamentally identical; *2)*, the increase in serum calcium resulting from parathyroid administration is not entirely or even largely due to the formation of a non-diffusible calcium-phosphate compound; *3)*, more careful consideration must be given to the following factors in attempting to interpret the changes in serum calcium which follow the administration of parathyroid extract: *a)*, the effect of hemoconcentration per se, *b)*, the increased serum protein concentration and *c)*, the possible state of unsaturation of protein with calcium under normal conditions.

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BLOOD CHEMISTRY OF SURVIVING PARATHYROIDECTOMIZED DOGS

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Although considerable attention has been attached to the treatment of tetany which occurs in the parathyroidectomized dog a few days after complete extirpation of these glands, very little work has been done on the factors which are responsible for the subsiding and gradual disappearance of this symptom complex. We felt that some attention should be paid to a study of the inorganic constituents of the blood of animals that had completely recovered from this tetany complex. Further, in connection with the more specific problem of the causes of lenticular cataract development in parathyroidectomized dogs, it was thought that analyses for blood sodium, phosphorus, potassium, magnesium, calcium, and chlorides should be made on these dogs which had survived the temporary period (30-60 days) after parathyroidectomy. Having in mind Jacque Loeb's classical experiments on the antagonism of salts, we were led to suspect that we would find in the blood of surviving parathyroidectomed animals in which the serum calcium is low, some change in the other cations, Na, K, and Mg.

EXPERIMENTAL METHODS

Healthy, normal male and female dogs were selected from the animal colony and after an adequate control period, a bilateral thyroparathyroidectomy was performed under morphine-ether anesthesia. A quarter grain morphine with a half mgm. atropine was administered one-half hour prior to operation. The thyroids were carefully removed and the thyroid vessels ligated and cut as close to the carotid artery as possible. All the dogs considered in this paper developed tetany (within 24 hours) in the immediate postoperative period when calcium salts were withheld.

Calcium lactate, shown by Luckhardt and Goldbert (1), Salvesen (2) and later by Dragstedt (3) to prevent postoperative parathyroid tetany and calcium gluconate (4) were given in 1.5 g. per kg. per day doses, in the food or by stomach tube, for 6 or 7 days, after which time the dosage was withheld until the animal developed tetany and the calcium salt was then given by stomach tube to relieve the tetany. This procedure was repeated every week until the animal no longer developed tetany, at which time all therapy was discontinued.

The constant diet was bread and meat, care being taken not to give too much of the latter. One group of dogs received bone meal in the diet, while the other group received no bone meal at any time. No difference in behaviour or blood chemistry could be detected in the two groups. When all symptoms of tetany

had disappeared from the dogs, they were considered to be in the 'survival' period. Blood samples were taken at convenient intervals for determination and the animals carefully watched for any signs of tetany. If any tetany did develop it was relieved by Ca therapy. In no case was a blood sample taken until after 12-18 hours had elapsed following the dog's last meal.

The analytical methods used were the following. Calcium was determined by the technique of Kramer and Tisdall (5), slightly modified. After the addition of ammonium oxalate the pH as adjusted to brom-cresol purple. The precipitated and centrifuged calcium oxalate was washed twice with saturated calcium oxalate solution in 0.5 per cent ammonium hydroxide. The third washing was with 2 per cent ammonium hydroxide. Titrations were made with N/200 $KMnO_4$. The permanganate factor was redetermined in each experiment by means of standard sodium oxalate.

For phosphorus the technique of Fiske and Subarrow (6) was used.

Sodium was determined by the technique of Kramer and Gittleman (7). After adding the potassium antimonate reagent, the sides of the tube were rubbed with a pyrex glass rod until a heavy precipitate was formed. Then 4 cc. of 80 per cent redistilled alcohol was added dropwise from a burette with constant stirring. The rod was rinsed down with 30 per cent alcohol and this same rod was replaced in the tube before titrating. The tube was covered and after standing 1 to 1½ hours, was centrifuged for 10 to 15 minutes. It was then inverted and drained on a filter paper for 5 minutes. The titration was carried out in the centrifuge tube, the volume having been made up to 10 cc. before titrating.

For potassium, the technique of Kramer and Tisdall (8) was used. After precipitation was complete the volume was made up to 5 cc. with water and 0.5 cc. of a 2 per cent suspension of caprylic alcohol in water added. The tube was rotated until the precipitate just began to rise and centrifuged at once for 10 to 15 minutes. The supernatant fluid was poured off by tilting the tube carefully so as not to let the precipitate run down the side of the tube. The tube was then drained and wiped with a filter paper and the washing repeated twice more. Very fine tipped centrifuge tubes were used to diminish the danger of losing precipitate.

Magnesium[1] was determined by the method of Denis (9); chlorides by the method of Wilson and Ball (10); pH by the method of Hastings and Sendroy (11); CO_2 by the method of Van Slyke and Neill (12). Care was taken to check all methods against known standards, and reagents were renewed when a change was advisable.

DISCUSSION

For space economy, only the experimental data on a few of the surviving dogs in these studies is presented. Table 1 shows the condensed protocols on normal and parathyroidectomized dogs during the survival period.

As has been shown by numerous workers, the serum calcium and inorganic phosphorus values of the normal dog show some variation, the calcium varying from 9-12 mg. per cent depending upon the method used, and the inorganic phosphorus from 3-5 mg. per cent. With the methods as used by us, the normal calcium figures are within the 10 to 11.5 range and the inorganic phosphorus in the 4 to 5.5 range.

[1] So slight was the magnesium variation in the sera of our surviving dogs that we considered it of not enough significance to continue its determination. This was likewise the experience of Weaver and Reed (20).

TABLE 1. ANALYTICAL VALUES IN MG. PER 100 CC. OF BLOOD.

Date	Ca	P	Mg.	Na	K	Cl as NaCl	Remarks
	10.7	4.6	—	—	—	—	Normal dogs 12 hr. after meal.
	11.4	5.3	—	—	—	—	Same.
	11.5	3.8	—	372	25.1	660	Same.
	10.7	5.4	—	347	21.9	648	Same.
	11.3	4.1	—	322	22.2	—	Same.
	10.7	5.4	—	347	21.9	648	Same.
	11.5	3.8	—	372	25.1	660	Same.
				Dog I			
							Parathyroidectomy 10/25/28
							Last tetany 12/1/28
12/17/28	6.1	7.6	2.2	—	—	—	No tetany.
12/28/28	6.8	—	2.2	—	—	—	Same.
1/21/29	7.3	7.0	2.0	436	19.0	—	Same.
3/ 9/29	10.7	6.5	—	405	25.3	633	Same.
5/13/29	9.3	4.3	—	335	23.4	618	Same.
6/ 5/29	7.6	5.4	—	396	22.6	633	Same.
6/25/29	10.2	5.3	—	392	22.9	635	
				Dog V			
							Parathyroidectomy 10/27/28
							Last tetany 11/26/28
12/18/28	7.8	4.5	1.8	—	—	—	No tetany.
12/28/28	8.0	4.4	1.9	—	—	—	Same.
1/19/29	7.8	—	1.9	—	—	—	Same.
1/21/29	7.4	5.7	1.8	414	22.0	—	Same.
2/11/29	5.3	7.2	—	294	24.0	—	Violent tetany.
2/11/29[1]	8.0	7.2	—	305	24.0	—	No tetany.
3/ 9/29	11.1	6.3	—	322	24.0	661	Same.
5/13/29	8.7	6.3	—	308	25.9	665	Same.
5/19/29	—	—	—	—	—	—	Tetany severe.
6/ 5/29	7.4	6.5	—	380	20.0	649	No tetany; pregnant.
6/25/29	11.3	5.6	—	391	21.9	668	No tetany.
7/23/29	6.4	6.3	—	321	23.2	628	Same.
7/26/29	6.5	6.4	—	315	22.9	628	Same.
				Dog 50			
							Parathyroidectomy 4/15/29
6/ 8/29	7.5	6.5	—	—	24.0	639	No tetany.
7/11/29	6.1	9.8	—	334	22.5	636	Same.
7/12/29[2]	6.1	9.4	—	332	23.4	638	Same.
				Dog 'Mex'			
							Parathyroidectomy 10/27/28
							No tetany after 1/5/28
1/19/29	7.7	3.1(?)	—	440	—	—	No tetany.
1/21/29	7.7	4.5	—	445	21.0	—	Same.
1/26/29	8.4	4.5	—	443	20.0	—	Same.
2/28/29	11.6	4.0	2.1	314	23.0	—	Same.
6/18/29	11.5	6.3	—	310	—	660	Same.

[1] Forty minutes after 10 cc. Ca gluconate (10%) intravenously.
[2] Blood pH, 7.37; blood CO_2, 51.8 vol. %.

Dog I after parathyroidectomy and during the tetany periods had shown the usual low Ca/high inorganic-P ratio and exhibited severe tetany in the test periods. After a period of nearly a month following the operation, and with

removal of all calcium therapy, no more tetany developed. A blood sample taken on 12/17/28 showed a low calcium and high inorganic phosphorus. There seems to have been a steady rise in blood calcium into March, when the value on 3/9/29 was 10.7 per cent with a small drop in phosphorus. The calcium values fluctuated in this dog, showing a drop on 6/5/29 with a subsequent rise on 6/25/29 to an normal value. The inorganic phosphorus, once down to a normal level, seems to have stayed down. The other values of Na, K, and chlorides at all times were within the normal range.

At the end of the test period in July this animal showed the characteristic lenticular cataract developments. At no time during the survival period was the animal in tetany and each day ate its food readily, gaining in weight.

Dog V, a female, showed violent tetany in test periods. Forty-five days after operation and 3 weeks after calcium therapy had been stopped, the blood picture showed a reduced calcium with normal inorganic phosphorus. This situation held true until 2/11/29, when a violent tetany ensued, the blood picture showing a markedly lowered calcium with a high inorganic phosphorus. Ten cc. of 10 per cent calcium gluconate solution injected in the saphenous vein alleviated the tetany and animal appeared normal. A blood sample taken 40 minutes later showed a rise of calcium to 8.0 but no fall of phosphorus. In this dog at this time apparently a raised calcium was more important in the alleviation of tetany than any other inorganic change. The Na and K showed no change. Inorganic phosphorus continued above normal in this dog until 6/25/29 when it dropped to 5.6, but subsequent analysis showed that a drop in calcium and a rise in phosphorus had taken place, with no development of any signs of tetany.

Dog 50, if one considers Ca/P ratio important, is perhaps the most interesting dog of the series. As with all other dogs in this study, distinct signs of hypoparathyroidism were always present when calcium therapy was removed in the pre-survival period. However, when the dog entered the survival period, its blood picture showed a low blood calcium and raised inorganic phosphorus. The K and chlorides were normal. A month later, 6/8/29, the blood picture showed a much lower calcium and the highest inorganic phosphorus noted in any dog in this study. These determinations were repeated, with pH and CO_2 the following day. The low Ca/P ratio still persisted, with normal values for the other determinations. At no time during the survival period did this dog show the slightest signs of tetany. It is unfortunate that the dog was sacrified for other studies so that more figures could not have been obtained on it.

Dog Mex, 26 days after last signs of tetany, showed a low blood calcium and normal inorganic phosphorus. There was a gradual increase in calcium level until by 2/28/29 the calcium and inorganic phosphorus were both at normal values. No tetany was ever observed in this dog after the survival period had set in.

Of the 14 parathyroidectomized dogs which survived, only 4 attained finally normal serum calcium values. In 2 of these, the phosphorus values were slightly higher than normal (6.0 to 6.3). The Na, K, and chlorides were in the normal range.

FACTORS INVOLVED IN SURVIVAL OF PARATHYROIDECTOMIZED DOGS

The abundance of experimental data of many workers has confirmed the early statement of Luckhardt and Goldberg that calcium salts given in proper amounts will control the tetany of parathyroidectomized dogs, and that these dogs, under this treatment, will after 30 to 60 days no longer need any calcium therapy and will live for 3 to 20 months, depending upon the individual characteristics of each animal. Care must of course be taken that the diet be restricted to a minimum meat requirement. Foods high in phosphate will tend to throw these surviving dogs into latent tetany (13). Pregnancy and lactation will necessitate extra precautionary measures, but latent tetany brought about by pregnancy and lactation can be controlled by proper calcium treatment. The matter then resolves itself into the question of just what factors are responsible for the absence of tetany during the survival period.

Greenwald (13), after determining the efficacy of cod-liver oil on parathyroidectomized animals, stated that the survival of parathyroidectomized animals "after dietary control has ceased indicates very strongly that there are accessory parathyroids in such animals or that some other tissue takes over the function of the parathyroid glands." In support of this argument Greenwald cited the fact that nearly all such surviving animals of Brougher (14), those of Swingle and Wenner (15) treated with strontium lactate, those of Wenner (16) treated wih magnesium lactate, and those of Wenner (17) treated with NH_4Cl, finally attained a normal calcium content in the serum.

The thesis of the presence of accessory parathyroid tissue in surviving parathyroidectomized dogs with no calcium treatment, showing normal serum calcium values, must be allowed for the present. We believe, however, that it cannot hold for surviving parathyroidectomized dogs, with no calcium treatment, showing serum calcium values at the admitted tetany level (4-7.5 mg. per cent). It would seem to us that some other factor must be involved here. The suggestion of Dragstedt (18) that "after a varying period of 4 to 6 weeks the nervous system becomes so adjusted to the altered blood that tetany no longer results when treatment is stopped" appears reasonable after a consideration of our data.

The tetany induced in rachitic rats by Hess, Weinstock, Benjamin, and Gross (19) by suddenly changing from a rachitic to a normal diet, with a fall in blood calcium, suggests that even in parathyroidectomized dogs in the survival period one must carefully watch so that no abrupt change is made in the dietary. It would appear that here we are dealing not alone with the function of the parathyroid gland to maintain a normal equilibrium between Ca and P, but with some other protective mechanism which, in the absence (or in the case of rickets, dysfunction) of the parathyroid gland, is inadequate when a sudden shift is made in the blood condition.

There was some indication from our data that, when the serum calcium was low (after parathyroidectomy), the serum sodium tended to be raised above the normal level. This is particularly evident if one considers the condensed protocol of dog 'Mex.' However, in the majority of cases there was no real correlation between a lowered calcium and a raised sodium serum value, so we have

concluded that there was no consistent significant change in serum Na, K, Mg and chlorides after parathyroidectomy during the survival period.

Our data lead us to the conclusion that some adjustment (other than accessory parathyroid hypertrophy) takes place in the parathyroidectomized animal so that it is able to withstand both the altered Ca/Na/K and Ca/P ratios. Only when the question of the rôle of these various inorganic salts in the metabolism of muscle and nerve has been worked out will one be able to state with any degree of accuracy just what part they play in the absence of tetany in survival after parathyroidectomy.

SUMMARY

A low serum calcium and high serum inorganic phosphorus, which according to some workers should precipitate tetany in the parathyroidectomized dog, does not do so in parathyroidectomized dogs in latent survival. In the dog after parathyroidectomy, serum calcium and inorganic phosphorus can remain at tetany levels for at least 9 months without returning to normal. There are no significant changes in sodium, potassium, magnesium and the chlorides after parathyroidectomy during the survival period.

The presence of accessory parathyroid tissue does not explain the survival of all parathyroidectomized dogs, since parathyroidectomized dogs may survive for at least 9 months with low serum calcium and high inorganic phosphorus. We are of the opinion that some neuro-muscular adjustment in the animal allows bodily functions to go on apparently normally, with tetany absent even though abnormal Ca/P and Ca/Na/K ratios are present.

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CARBOHYDRATE METABOLISM STUDIES IN HYPOPHYSECTOMIZED RATS

I. THE RELATION OF VARIOUS METHODS OF GLUCOSE ADMINISTRATION TO THE BLOOD SUGAR CURVE

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Sugar tolerance cruves in hypophysectomized animals have yielded a variety of results. Goetsch, Cushing and Jacobson (1), and Sachs and MacDonald (2) reported an increased tolerance in hypophysectomized dogs given glucose by mouth. Corkill, Marks and White (3), using rabbits given 0.5 gm. of glucose by mouth, found that most animals showed an increased tolerance, although in a few no marked differences were observed before and after hypophysectomy. No statement was made of the length of time intervening between the operation and the tolerance test.

Kepinov (4) likewise has reported a lower average blood sugar curve in 6 hypophysectomized dogs fed glucose than he found in the same animals prior to operation. Zwarenstein and Bosman (5) report that the blood sugar did not rise to the same extent as in normal controls half an hour after injection of glucose into the dorsal lymph sac of hypophysectomized toads (Xenopus laevis). Mahoney (6) gave a hypophysectomized chimpanzee 1 gm. of dextrose, per kg. body weight, intravenously and found a lower blood sugar curve than was found in the same animal under similar conditions before the operation.

Pickat (7) extirpated the pituitary in dogs and reported a transient glycosuria 1 to 3 days after the operation, followed by an increased tolerance during the first post-operative months. After this the tolerance returned to normal. D'Amour and Keller (8) obtained prolonged high curves within 1 to 14 days after hypophysectomy or damage to the region of the hypothalamus. In those animals that were followed, the curves returned to normal in 9 to 20 days. No detailed data were given and the number of animals in some groups was small.

Colwell (9) and Houssay, Hug and Malamud (10) determined the effects of continuous intravenous glucose injection in dogs on the urinary excretion of sugar. They found no significant variation from normal after pituitarectomy.

Biasotti (11) has published a summary of his experiments on 14 hypophysectomized dogs given a single dose of glucose intravenously. He found that the blood sugar rose to higher values and returned to normal more slowly

[1] Aided by grants from the American Association for the Advancement of Science and the Committee on Scientific Research of the American Medical Association.

than in his group of controls. On the other hand when glucose was given by stomach tube, the hypophysectomized dogs gave an average curve slightly more flat than that of the normal animals.

Thus we find that, in general, when glucose is given by stomach tube to hypophysectomized dogs or rabbits, the blood sugar curve is lower than normal

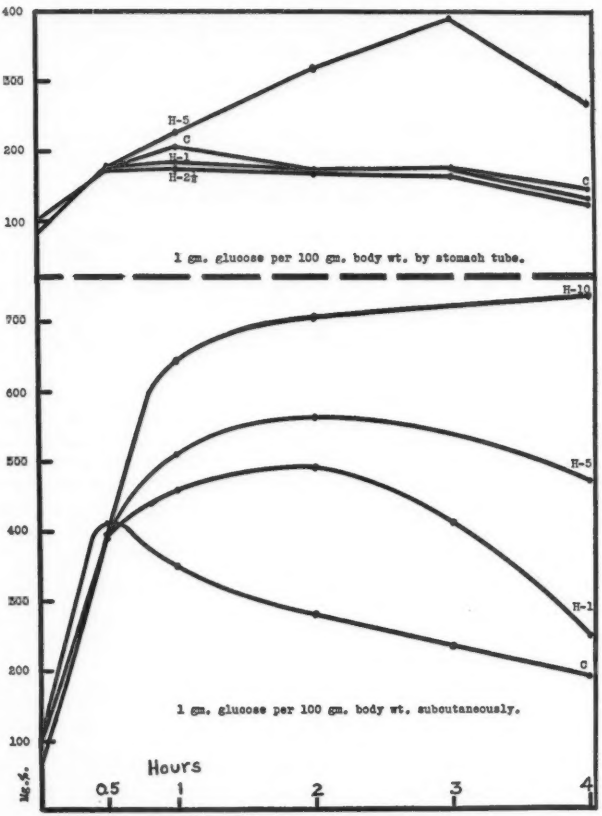


Fig. 1. *C*, controls (normal animals); *H*-1, *H*-2½, *H*-5, *H*-10, hypophysectomized rats at 1 to 10 weeks after operation.

shortly after the operation but subsequent tests may correspond to the preoperative curve. If, however, a single injection of glucose is given intravenously the tolerance seems to be low. The available data on continuous intravenous injection differ.

In 1932 we commenced a study of the blood sugar curves in hypophysectomized rats in an attempt to explain convulsive deaths occurring in another experiment. The curves which we obtained showed the same variation with the method of administration of glucose which has recently been reported by Biasotti (11) as mentioned above. This led us into the following work reported

in this paper. *1)*. A comparison of the curves obtained by alimentary, subcutaneous, and intraperitoneal injections. *2)*. Observations on the rate of absorption of glucose from the alimentary tract. *3)*. A correlation of these results with the curves obtained following the subcutaneous injection of 3 different amounts of glucose.

METHOD

Male rats 4 to 6 months of age were hypophysectomized by the neck approach, or reserved as normal controls. All animals were fasted 24 hours prior to use. To some a freshly prepared 50 per cent glucose solution was admin-

TABLE 1. BLOOD SUGAR VALUES AS MG. PER 100 CC. IN RATS FASTED 24 HR. AND AT INTERVALS FOLLOWING 1 GM. GLUCOSE PER 100 GM. BODY WT. BY STOMACH TUBE.

Fast.	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.	Fast.	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Normal Controls						*Hypophysectomized 1 week*					
112		270	346	176		89	312	234	234		191
81		205	156	189		77	98	117	256		96
131		238	135	173		86	189	196	131		113
109		184	149	159		92	126	164	134		103
95		324	149	195		72	163	172	141		116
89	127	140	150	168	156	76		179	215		189
108	252	128	157	169	154						
91	141	134	142		119						
102	173	203	173	176	143	82	178	177	185		135
Hypophysectomized 2½ weeks						*Hypophysectomized 5 weeks*					
					5 hr.						5 hr.
68		162	181	216		78		410	410	465	249
74		286	135	119		73		308	340	554	400
73		205	159	135		95		140	350	500	235
73		243	151	135		75		160	320	400	290
100		125	200	250	125	80		150	315	250	220
118		145	150	140	100						
105		125	170	135	110						
100		160	200	180	145						
95		125	215	165	130						
89		175	173	164	122	80		234	347	434	279

istered by stomach tube in the amount of 1 gm. per 100 gm. body weight, while to others a 20 per cent solution was given by subcutaneous or intraperitoneal injection. Doses of 0.2 gm., 0.5 gm., and 1 gm. per 100 gm. body weight were given subcutaneously in the loose skin at the nape of the neck and back, care being used to avoid loss of solution after withdrawing the needle. An attempt was made to distribute the solution by manipulation. Only the 0.5 gm. per 100 gm. body weight dose was used in the intraperitoneal injections. Blood samples were obtained in most instances while fasting and at ½, 1, 2, 3, and 4 hours after glucose was given. The tail was clipped, the first drops of blood discarded, and the sample taken directly into a micropipette. This was discharged into a measured quantity of tungstic acid. The centrifugates were assayed by the Folin ferricyanide method.

In the case of the alimentary absorption studies the animals were anesthet-

ized with amytal or nembutal several minutes prior to the time the gastro-intestinal tract was removed. When the tract was dissected free it was immediately dropped into a beaker of boiling water to inactivate enzymes, and then cut into small pieces with clean scissors. After cooling the contents of the beaker were transferred quantitatively to a volumetric flask where proteins were precipitated by the Somogyi zinc sulphate method. After filtration and appropriate dilution, samples were analysed for sugar by either the Folin

TABLE 2. ALIMENTARY ABSORPTION OF GLUCOSE IN RATS AFTER ADMINISTRATION OF 1 GM. PER 100 GM. BODY WT.

	Controls	Hypophysectomized 1 wk.	Hypophysectomized 2½ wk.
1 hr. absorption			
No. of animals	8	7	
Mean % absorbed	38	23	
Std. Dev. of mean	±2.4	±2.2	
2 hr. absorption			
No. of animals	7	7	5
Mean % absorbed	56	35	37
Std. Dev. of mean	±3.2	±1.9	±2.3
4 hr. absorption			
No. of animals		5	
Mean % absorbed		57	
Std. Dev. of Mean		±2.6	

COMPARISON OF DIFFERENCES IN ALIMENTARY ABSORPTION OF GLUCOSE.

Groups compared	Difference of means	Std. dev. of difference	Ratio of difference to std. dev. of difference
Absorption at 1 hr.			
Normal controls with hypophysectomized 1 wk.	15	3.3	4.5
Absorption at 2 hr.			
Normal controls with hypophysectomized 1 wk.	21	3.7	5.7
Normal controls with hypophysectomized 2½ wk.	19	3.9	4.9
Hypophysectomized 1 wk. with hypophysectomized 2½ wk.	2	3.0	.7

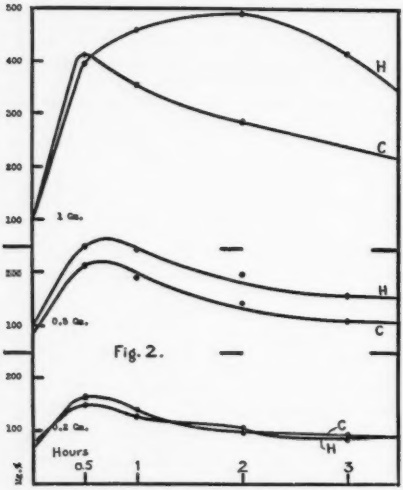
ferricyanide method or the Somogyi modification of the Schaffer-Hartmann procedure.

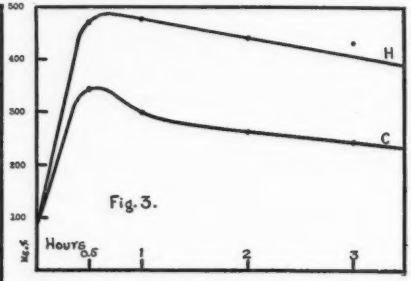
RESULTS

It will be seen from figure 1 that the average blood sugar curves following 1 gm. of glucose per 100 gm. body weight by stomach tube are not significantly different from the normal at the periods of 1 and 2½ weeks after hypophysectomy, while at 5 weeks the curve is distinctly 'diabetic' in type. Table 1 presents the individual values.

In contradistinction to the findings on alimentary administration, the curves of figure 1 are significantly higher than normal 1 week after hypophysectomy where the same dosage of glucose was given subcutaneously.

Obviously some factor affects the transfer of sugar between the lumen of the gastro-intestinal tract and the blood stream which does not influence the passage from the subcutaneous tissues to the blood. Initial observations showed a deficient rate of absorption from the gut. Phillips and Robb (12) in 1934 published the same findings in a group of rats 15 to 60 days after hypophysec-





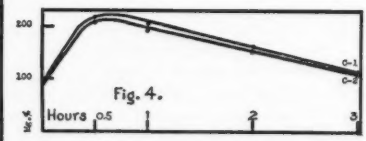


Fig. 2. Glucose injected subcutaneously in the dosages shown per 100 gm. body wt. *H*, hypophysectomized 1 week; *C*, normal controls.

Fig. 3. Glucose intraperitoneally, 0.5 gm. per 100 gm. body wt. *H*, hypophysectomized 3½ weeks; *C*, normal controls.

Fig. 4. Glucose subcutaneously in normal rats, 0.5 gm. per 100 gm. body wt. *C*-1, diet restricted to two-thirds normal; *C*-2, unrestricted diet. A 24-hr. fast preceded tests in each group.

tomy. The deficiency was 30 per cent in their group. This has been confirmed by Bennett (13) who found a decrease of 35 per cent in absorption several weeks after hypophysectomy. We have attempted to define the development of this deficiency somewhat by observing groups of animals 1 week and 2½ weeks after hypophysectomy.

The results (table 2) indicate that there is as much deficiency in rate of absorption 1 week after hypophysectomy as exists 2½ weeks following operation. In our series it is 36 per cent. When statistical comparisons are made between the controls and hypophysectomized groups the differences are significant. However, when we compare the group 1 week after hypophysectomy with the 2½ week group we find figures which nearly coincide. Evidently the rate of absorption for glucose decreases rapidly after pituitary removal and within the first week reaches a level about 36 per cent lower than normal which is then maintained. The rapidity with which this change occurs argues in favor of a direct effect of pituitary removal.

Following these findings it became of interest to determine whether

amounts of glucose comparable with those actually absorbed from the gastro-intestinal tracts of the above animals, when given subcutaneously, would be compatible with a normal blood sugar curve. Figure 2 indicates that when only 0.5 gm. per 100 gm. body weight is injected under the skin of hypophysectomized animals the resulting curve is only slightly higher than that of the controls. When the dosage is reduced to 0.2 gm. per 100 gm. body weight both groups handle the sugar equally well. The deficient rate of gastro-intestinal absorption becomes, then, an adequate explanation for the differences observed between the two methods of glucose administration.

In order to explain the change from the apparently normal curves obtained at 1 and 2½ weeks after pituitary removal when the glucose was given by stomach tube, and the very high curves obtained in the same way in animals hypophysectomized 5 weeks and longer, we studied the influence of time after operation on the blood sugar curve following subcutaneous injection. Figure 1 gives the curves obtained when the same dose of glucose was given 1, 5 and 10 weeks after operation. They show a progressively decreasing ability to remove excess glucose from the blood. This gradual reduction in tolerance suggests an indirect effect of pituitary deficiency, perhaps through an influence on the liver.

Length of fast and previous carbohydrate intake are known to affect the blood sugar curve in normal animals. Since our hypophysectomized animals ate but slightly more than two-thirds the amount of foods consumed by the controls, we limited some of the latter individually to this amount for a period of 2 weeks, when tolerance tests were made (fig. 4). While the average for the group of normal animals with limited food intake was slightly higher than that for the group without limitation the difference is insignificant when compared to that caused by hypophysectomy.

Groups of hypophysectomized and normal animals were observed after intraperitoneal administration of glucose to rule out possible variations in absorption which might occur after subcutaneous injection. After giving 0.5 gm. per 100 gm. body weight these groups showed a similar relative difference 3½ weeks after pituitary removal (fig. 3). The individual curves, however, showed no greater uniformity. Apparently then, variation in absorption was not significantly greater on subcutaneous injection than it was by the intraperitoneal route.

SUMMARY

Rats hypophysectomized 2½ weeks or less, fasted 24 hr. and given 1 gm. of glucose per 100 gm. by stomach tube yielded curves similar to those found in normal animals with the same dose. However, when this amount of glucose was given by stomach tube 5 weeks after operation a high curve of diabetic type resulted. In contradistinction to alimentary administration if the above dose or one-half the amount was given subcutaneously, abnormally high sugar curves resulted as soon as 1 week after hypophysectomy. This inability to remove excess sugar from the blood progressively increased in severity for a period of several weeks. This suggests an indirect effect of pituitary removal.

A decrease of 36 per cent in the rate of intestinal absorption of glucose explains why the blood sugar curves in the animals which received the sugar in this way did not show higher than normal curves during the first 2½ weeks following hypophysectomy. This deficiency of intestinal absorption for glucose appears abruptly after pituitary removal and once it is established, remains constant. This suggests a direct effect of pituitary deprivation.

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CASE REPORTS

A STUDY OF THE METABOLISM OF A DWARF UNDER TREATMENT WITH GROWTH HORMONE

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The effects of administration of growth hormone of the anterior hypophysis to lower animals have been well established since the production by Evans and Long (1) of experimental gigantism in rats. Since that time efforts have been made by several investigators (2) to administer preparations of the growth-promoting substances in cases of human infantilism. Examination of the reports of these investigations shows that, while in certain selected cases growth may be stimulated beyond that previously attained by the patient, and in some cases beyond the theoretical normal for the age of the patient, yet the results are not as striking as had been hoped. Evans (3) and Novak (4) have both commented on this and have concluded that the chief reasons for the disappointing results are the low yield of hormone from the glands of cattle, the instability of the product, and the lack of a source of pure, or at least more concentrated, material. It seems apparent also that there is still much to be learned about the clinical use of pituitary extracts in replacement therapy. It may be concluded, however, granting that true hypophyseal infantilism exists in a particular patient, that there are at least two definite contraindications for the use of growth hormone, closure of the epiphyseal disks, and sexual maturity. It is universally stated in the reports that either skeletal or sexual maturity prevents action by the growth hormone and, since sexual maturity leads to skeletal maturity, it is essential that the preparation used to be free from gonadotropic substances. It has been noted also that simultaneous administration of thyroid and growth hormones gives results superior to those obtained with the growth hormone alone. The exact reason for this is not known.

The mechanism of the action of the growth hormone is still unknown. Since the hormone affects growth, it has been natural to expect a retention of nitrogen during its administration. Several authors have approached this problem from different viewpoints. Bierring and Nielsen (5) found a significant increase in the nitrogen content of the soft tissues of rats receiving growth hormone. Lee and Schaffer (6) obtained similar results, and showed also that the weekly excess gain in weight of the injected animals over their litter mate controls closely parallels the excess nitrogen retention determined by balance experiments. Their data indicate that the nitrogen content of the soft tissues

and the fat-ash-free dry tissues are the constituents most specifically influenced by the hormone. Agnoli (7) found that anterior lobe extracts promote deamination of glycocoll by the blood. Gaebler (8) found that a single injection of hormone in dogs causes an immediate and marked fall in urinary nitrogen, due chiefly to decreased urea excretion, while the fecal nitrogen remains unchanged. The earlier work of Teel and Watkins (9) indicates that this effect, which lasts for several days, is not due to renal damage since the nonprotein nitrogen of the blood is actually decreased during this interval. All these facts point to anabolism of protein as the chief effect of the hormone in soft tissues.

We present here the results of a study of a dwarf treated with growth hormone.[1]

History. The patient, a woman, aged 24, on admission to the Psychiatric Institute on Nov. 21, 1934, according to information obtained from the father and a sister, had developed normally until about the age of 10 years. She had had the usual diseases of childhood, none of them severe or resulting in noticeable sequelae. In 1918, when the patient was 9, she was said to have had influenza which was accompanied by fever up to 104°F., and coma or severe stupor during the first 3 days of the illness which lasted for 2 weeks. Neither informant nor the physician who attended her at this time could recall any other symptoms indicative of encephalitis, such as diplopia and headache. Menses were never established, and growth apparently did not occur after this period. The patient graduated from grammar school at 12 and started high school, in which she continued until the third year. Reports from the school confirmed the relatives' statements that school achievement and social adjustment were apparently average until about the third year in high school when she almost suddenly became reticent, withdrew progressively from social contacts and failed in school work. She stopped going to school,

[1] This material was kindly furnished by Parke Davis and Company, Detroit, Michigan.

became difficult to manage because of sudden outbursts of violent behavior, even toward the father to whom she previously had been much attached. In the 8 or 9 years prior to commitment to the Psychiatric Institute, and after the appearance of the change of behavior, she was observed in several clinics and was committed to several state hospitals. Various classifications were made of the illness, such as hebephrenic dementia praecox and psychosis with endocrine disturbance.

Physical Examination. The patient was 64.5 inches in height, weighed 82.5 lb., and was well nourished, with the body proportions of a preadolescent child but with facial features much older in appearance. There was absence of palpable glandular tissue in the fat of the mammary regions, complete absence of axillary hair, a downy growth of hair on the upper lip, and a slight growth over the pubic region. The findings in the heart indicated rheumatic mitral stenosis and insufficiency. The external genitalia were infantile, as was the cervix uteri. The internal genitalia were not palpable. Neurologic examination gave negative findings.

Roentgenograms of the skull and other parts of the skeleton revealed an anomalous configuration of the sella turcica, lack of fusion of the epiphyses of the hand and of the crest of the ilium, although those of the elbow, knee and hip indicated ossification consistent with an age of from 18 to 25 years. Encephalography showed normal ventricles but an increase of air in the subarachnoid space. The blood pressure was 128 mm. systolic and 86 mm. diastolic. Examination of the blood and spinal fluid revealed no changes from the normal in cellular constituents, serology or chemical values. Urinalyses on several occasions gave negative results. The B.M.R. on 3 different occasions during a 3-month period ranged from —7 to +9 per cent.

A battery of both verbal and nonverbal psychometric tests revealed an I.Q. of less than 30, the mental age being 4 or 5 years. Her general behavior on the ward was more or less uniformly quiet, apathetic, showing practically no spontaneity either in speech or motion.

Diagnosis. The clinical impression from these observations was psychosis due to metabolic endocrine disease, with rheumatic heart disease. The impression of the internist was that of a deficiency in both growth and sex hormones of the anterior pituitary gland.

Endocrine Studies. On June 16, 1935, after two 4-day periods as controls, administration of growth hormone was instituted, the dose being 20 R.U. daily, given subcutaneously. This was continued for 35 days, a total of 700 R.U. being given. The only change in the patient's condition noticed during this period was an increase of 3 lb. in weight. No medication was given for the following 36 days. Again the only clinical change was a further gain of 2.5 lb. in weight. Administration of the same amount of growth hormone was again instituted and continued for 37 days, and in addition 200,000 I.U. per day of vitamin D[2] was given during the last 23 days of this period. The vitamin D was given in the hope that some change might be instituted in the bone metabolism so that skeletal growth could be renewed. During this interval the patient gained an additional 5 lb. in weight, no other changes being noticed. The study was continued for 7 days after all medication had been stopped. Two days later acute appendicitis developed and an emergency appendectomy was performed. The patient was apparently recovering when she died without warning 2 days after the operation.

Autopsy. This was performed about 60 hours after death. The anatomic diag-

[2] The vitamin D solution contained 1,000,000 I.U. per gram, and was furnished by the Mead Johnson Company.

nosis was: 1. Mitral stenosis and parenchymatous degeneration of the heart. 2. Recent surgical removal of the appendix. 3. Low grade peritonitis in the lower right side of the pelvis and appendiceal region. 4. Congestion of lower lobes of both lungs. 5. Severe hypoplasia of the ovaries. Grossly, on the right side, near the middle portion of the fallopian tube, there was a remnant of very soft tissue, with no parenchymatous tissue present. On the left side no trace of an ovary was present. 6. Infantile uterus. 7. Softening of the spleen. 8. Parenchymatous degeneration of the brain, kidneys, liver and adrenal. Conclusion: Death was probably due to circulatory failure, with generalized toxemia.

Histopathology. The thymus showed an early stage of involution, with considerable fatty infiltration and arteriosclerosis. The lining epithelium of the thyroid acini was vacuolar and cuboidal. The acini were incompletely filled with colloid, and in some instances contain no colloid; they were of fairly uniform size. The trabeculae of the thyroid were of average size and contained thin-walled blood vessels which were distended with red blood cells and pigment-filled macrophages. There were focal accumulations of round cells and occasional cells throughout the fibrous reticulum. The picture was one of a physiologically active gland, giving the impression of a gland characteristic of about puberty. A few small areas suggested a fetal thyroid. Grade II hyperplasia.

Merely the cortex of the ovary was present, with a pronounced infiltration with round cells; the medulla was replaced by fat, fibrous tissue and scattered tubular structures lined with columnar epithelium. There was no structure that could be interpreted as a Graafian follicle. The gland was undeveloped, with atrophy and disappearance of Graafian elements.

No important abnormalities of the adrenals were found. There was a pronounced passive hyperemia in the hypophysis. A large colloid-filled cyst, lined with low cuboidal cells varying in depth from 1 to 3 layers, was present in the pars intermedia and there was several other cysts of various sizes in the pars intermedia and the pars anterior. The remaining cells in the pars intermedia were in nests, with vacuolated vesicular nuclei of moderate size. In some instances the centers of the nests were occupied by polyhedral cells of the same general character. A close interrelationship between these nests, the cysts and the cells of the anterior pituitary could be traced.

The brain showed somewhat flattened convolutions and was hyperemic, with distended blood vessels; there were no opacities, hemorrhages or other lesions. The basilar arteries were collapsed and soft to the touch. The cerebellum was of normal size. Petechial hemorrhages were numerous in the caudal portion of the optic thalamus. The lateral ventricles were moderately dilated; the third ventricle was much more so. There were hemorrhages in the white substance of the cerebellum. The outstanding microscopic changes in the brain were stasis or edema of the cerebral tissues, especially in the cortex; swollen oligodendroglia; and aseptic meningitis. Ganglion cell changes were present but mild; they showed only occasionally as neuronophagia. The changes were significant of a toxic condition.

The results of the metabolic study are shown in the figures. Figure 1 shows the nitrogen balance. Almost immediately after the administration of growth hormone was instituted the food intake increased nearly 100 per cent, and the daily retention of nitrogen even more. After administration of the hormone was stopped the food intake and nitrogen retention fell somewhat, although neither returned to the level of the control period. During the second period of

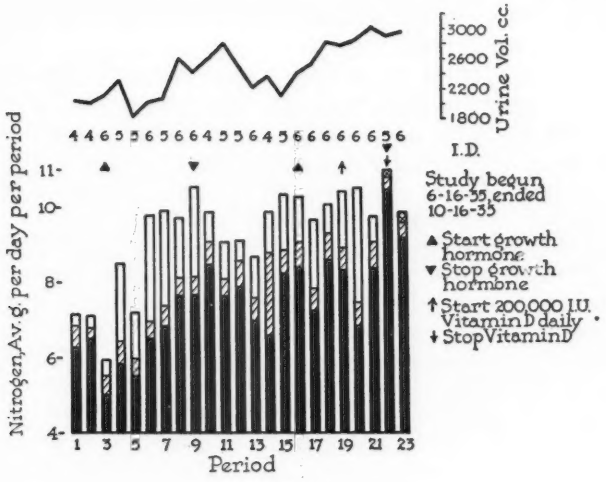

Fig. 1.

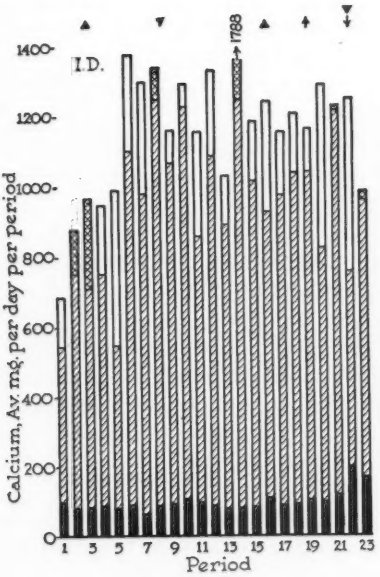

Fig. 2.

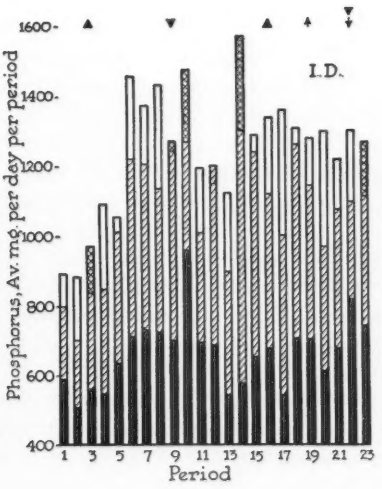

Fig. 3.

The indicia are explained in figure 1.

administration of the growth hormone, the intake again rose slightly, but the nitrogen retention did not become as great as during the first period of administration. This may indicate the development of a physiologic immunity to the hormone. During the final 2 periods the nitrogen balance became slightly

negative, but in view of the appendicitis which developed shortly after it is hard to state the cause of this.

Figures 2 and 3 show the calcium and phosphorus balances, respectively. There was no marked change in calcium balance at any time, although there was a possible tendency to greater retention during the periods when growth hormone was being administered. It is of interest, however, that the overall balance for both calcium and phosphorus was positive. There is no demonstrable relationship of phosphorus balance with treatment.

The chemical methods used were slight modifications of standard procedures. All food eaten by the patient was weighed, and duplicate samples were weighed and sent to the laboratory where each day's intake was kept in the refrigerator until all was collected. Nitrogen determinations were made in duplicate on the well mixed specimen by the Kjeldahl technic. Urine and feces were mixed with sufficient concentrated sulphuric acid to render them strongly acid immediately after being voided. Kjeldahl nitrogen determinations were made on these daily. The food was then dried in an oven at 140-150C., and ground to a fine powder and mixed. A portion of this was ashed in a platinum dish. Samples of urine and feces were also ashed in platinum dishes after being evaporated to dry residues over small flames. The ash was dissolved in 0.5N HCl, the solution diluted to 200 cc., and aliquots used for determination of Ca and P. The methods were slight modifications of the method of Shohl and Pedley for calcium, and of Pincus for phosphorus (10).

COMMENT

The case presents a confusing picture. Clinically, the impression was pituitary dwarfism complicated by several factors. The pathologic study, on the other hand, shows that, with the exception of the adrenals, the hypophysis was macroscopically and microscopically the most nearly normal of the endocrine glands. The impression of the pathologist was general involvement of the endocrines. While not in actual disagreement, the two impressions certainly are not in as complete agreement as might be wished. Among the several questions which might be raised are two. First, can endocrine dwarfism be readily recognized clinically in all or nearly all cases? Second, in this general type of disease, in which it is considered that one or more of the glands are under active or over-secreting, will pathologic examination of tissues be of service? That is, will a gland which is not functioning normally necessarily always show macroscopic or microscopic changes?

So far as we are aware neither question can be definitely answered in the affirmative, and it is this which we believe may be a contributing factor to some of the disappointments experienced in studies of human infantilism.

No growth of the bones could have been expected in the short period of study of this patient. On the other hand, the tacit assumption has been made by many investigators that the chief, if not the sole, lack in pituitary infantilism is deficiency of growth hormone; that supplying this initiates the process of development physically and mentally. In our patient no change was observed other than increased weight concurrent with high retention of nitrogen.

SUMMARY

Growth hormone of the anterior pituitary was administered to a dwarf, and a study made of the nitrogen, calcium, and phosphorus balance before, during and after the treatment. Nitrogen was retained, but calcium and phosphorus balances remained essentially unchanged during and after the administration of the hormone. An increase in weight was the only change noted in the patient's condition.

The authors wish to express their gratitude to Dr. C. I. Reed for aid during the work and to Drs. C. I. Reed and H. D. Singer for reading the manuscript. Thanks are also due to Drs. G. B. Hassin and George Milles for histologic studies.

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TRANSPLANTATION OF ADRENAL TISSUE IN ADDISON'S DISEASE[1]

MAX A. GOLDZIEHER AND S. B. BARISHAW

The following is the report of a case of Addison's disease in which transplantation of cortical tissue was successfully accomplished and the death of the patient 9 months after the operation made possible a study of the transplanted tissue and the patient's own adrenal glands.

CASE HISTORY

Reginald T. was born in 1908. His early history included scarlet fever at the age of 11, appendectomy at 14, tonsillectomy at 17 and turbinectomy at 19. He had frequent colds and sore throat. His present illness began early in 1932 with weakness, dizziness, fainting spells, lack of energy, nausea, vomiting and bronzing of the skin. A diagnosis of pernicious anemia was made and the patient was referred to the Hackensack Hospital, N.J., in Nov. 1933, and later to St. Luke's Hospital, N.Y.

At admission he seemed to be chronically ill, although fairly well-nourished, with a peculiar brownish discoloration of the skin. Folds and areas exposed to mechanical irritation were especially darkly pigmented. There was no pigmentation of the mouth. The blood pressure was 90/40. X-ray examination of the chest and adrenal region gave negative findings. The basal metabolic rate was —2 per cent. The electrocardiogram gave normal findings. The alcohol test meal showed no free HCl even after histamine. The urine was normal except for faint trace of albumin. The blood sugar was 100 mg. per cent and the urea-N 38.5 mg. per cent.

The patient was given injections of eschatin and saline intravenously and table-salt by mouth. He left the hospital after 2 months greatly improved; he was advised to take 4 drams of salt and 1 cc. of eschatin daily. He was readmitted 7 months later in a condition similar to that at his first admission. He had taken less salt than instructed, because of nausea. He again responded to intravenous therapy and was discharged improved with the same instructions. He returned a third time in Jan., 1935, and was treated as before for a period of 6 weeks, again with favorable response. He was discharged and advised to keep up the large amount of table-salt orally a well as daily injections of eschatin.

On March 3rd, 4 days after discharge, the patient appeared at Dr. Barishaw's office complaining of recurrence of his symptoms in spite of rigorous adherence to instructions. He was given daily injections of Goldzieher's interrenin (1, 2) for 1 week. He responded so well that 3 injections weekly thereafter were sufficient to maintain him in a comfortable condition. He was advised that his improvement could be maintained only by continued injections of the cortical extract. At the patient's suggestion of adrenal tissue transplantation he was referred to the Crown Heights Hospital where Dr. Koster performed the operation on March 29, 1935. The

[1] Read before the Twentieth Annual Meeting of the Association for the Study of Internal Secretions, Kansas City, Kansas, May 11, 1936.
A report of the autopsy findings and histological details were presented by Dr. F. H. Hemsath before the New York Pathological Society, May 28, 1936.

material was obtained from a female patient who was undergoing unilateral adrenalectomy for the treatment of hypercortico-genital syndrome. Through a midline incision, under local anesthesia, 30 or more thin slices of the adrenal tissue were placed in small pockets within the rectus muscle or between muscle and fascia. The grafts were immobilized by sutures and the abdominal wound closed.

The patient's condition at the time of admission was fair. His weight was only 130 lb. and his blood pressure 90/60. Before the operation he was given several cc. of interrenin and these injections were continued during his stay in the hospital. The postoperative course was uneventful. The blood pressure rose on the first day after operation to 105/70 and fluctuated thereafter between 98/65 and 100/70. Increase in physical strength, improved appetite and well-being were at once noted. He was discharged from the hospital after 11 days and was advised to take injections of an

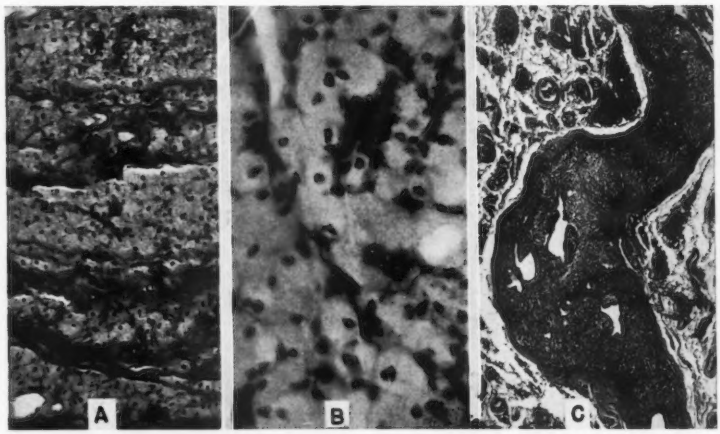

Fig. 1. *A*. Transplanted adrenal cortical tissue, low magnification. *B*. Same, high magnification, showing proliferation of nuclei and numerous giant cells. *C*, Atrophic adrenal of medullary tissue only. The narrow subcapsular seam consists of fibrous tissue which occupies the place of the absent cortex. Cross-sections of the large veins are gaping.

anterior pituitary extract, intended to stimulate the transplant by virtue of its adrenotropic factor.

The subsequent improvement was remarkable. Strength was regained and the bronze color lost. The weight increased to 144 lb. Six months after the transplantation the patient decided to go back to work. At that time he was not taking the table-salt nor any cortical hormone. He was well until October 8 when suddenly he fell ill and returned to the Crown Heights Hospital for observation for a period of 12 days. At admission his blood pressure was 90/55; it rose during the period of hospitalization to 125/60, dropped again as low as 80/40 and increased again to 105/60 at the time of his discharge. He was given liberal amounts of sodium chloride and a high vitamin C diet, to which he responded well. Parallel with the clinical improvement and gain in strength there was a significant reduction of his blood urea-N from 33 mg. per cent at admission to 21 mg. The fasting blood sugar was 90 mg. per cent, and the sugar tolerance curve was flat in type. The red-cell count was 4,600,000 with hemoglobin 93 per cent, lymphocytes, 42 per cent; oxygen consumption rate, 90 per cent of prediction; and the specific dynamic action to a standard protein meal, 10.

The patient left the hospital October 20 in good condition and remained well until shortly before his death. He was admitted in practically a moribund condition to the Christ Hospital, N.J., and died December 3.

An autopsy was performed by Dr. Hemsath who found a bronchopneumonia, a hypoplastic droplet-shaped heart and a narrow over-elastic aorta. There was no evidence of tuberculosis anywhere. The adrenal glands were both unusually small and rather difficult to find in the surrounding fat and connective tissue to which they were adherent. The site of the operation revealed within the tissue of the rectus muscle a string of nodules light to dark yellow in color. They were carefully excised and histologically examined together with the adrenal glands.

The implants showed persistence of cortical tissue, well-preserved and in good morphological condition. The cell outlines were sharp, the cytoplasm contained granulations and lipoid material which stained with the usual fat stains and proved to be anisotropic like the lipoids of the adrenal cortex. The nuclei were not shrunken and seemed capable of division as witnessed by a number of multinucleated giant cells, comparable to those frequently seen in adrenal cortical adenomas and carcinomas. The absence of interstitial fibrosis or round-cell infiltration is additional proof of the good survival of the transplanted tissue, since resorption of transplants is known to be accompanied by inflammatory cell infiltration and then by reparatory fibrosis and scar formation.

Microscopic examination of the adrenal glands revealed total absence of the cortices. The place of the cortical tissue was indicated by a seam of fibrous tissue which enshrouded the centrally located remnants of the adrenal medulla. The latter was morphologically easily recognizable, although the cells seemed to be rather small, atrophic and poor in cytoplasm. Whether or not they contained phaeochrome material could not be ascertained.

DISCUSSION

Summarizing these findings, it can be stated that the adrenal glands of this patients were in such a state of atrophy that they could not have functioned at the time or quite some time before death. This applies particularly to the cortex which appeared to be completely substituted by scar tissue. This type of adrenal lesion is well known in the literature and spoken of as simple atrophy, sclerosis of the cortex, essential or cytotoxic atrophy. The variety of terms reveals our ignorance of the cause and nature of this lesion. Evidence of inflammation is usually absent and rules out an infectious origin. The sometimes postulated cytotoxic pathogenesis is not supported by facts; more plausible, in the light of recent researches, is an absence or inadequacy of the pituitary adrenotropic principle with resultant atrophy of the gland and superimposed necrobiotic changes. However, the anatomical appearance of the adrenals together with the clinical history, proves that these glands were not functioning at least for some time prior to death, hence the survival was dependent for a time upon the hormones present in the injected extracts. As he had not received injections of the cortical extract in the period preceding his death it appears that the transplanted cortical tissue was capable of meeting his hormone requirements fairly adequately. The marked improvement of the patient after the implantation, including higher blood pressure, increased muscular strength and loss of pigmentation, together with other more

subjective signs, is the clinical evidence as to functional ability of the transplanted cortical tissue, and if morphological appearance is any criterion of function, the transplanted tissue was capable of producing cortical hormone. The good state of preservation in which the transplant was found, about 9 months after the operation, proves that the clinical improvement was not merely dependent upon the hormones present in and resorbed from the graft. It seems safe to assume that transplanted tissue which had been functioning for a period of 9 months and which showed no sign of decay and but little evidence of regression at the time of death, might keep on functioning indefinitely and if present in sufficiently large mass, might be enough to satisfy all ordinary hormone requirements of the body.

Thus transplantation seems to be a promising procedure in the treatment of Addison's disease and might, under improved conditions, approach a real cure. Previous experiences, both in animals and in man have commonly been unfavorable.

Early experiments (3, 4, 5, 6, 7, 8, 9) were unsuccessful. Mainly frogs and rats were used. The implants were made into the subcutaneous or muscular tissue, the peritoneum, the kidneys, spleen and liver. Successful transplantations in frogs and rats were reported by Abelous (9), Poll (10) and Schmieden (11). Most of the grafted tissue, however, became necrotic and only parts of the cortex survived while the medullary tissue became entirely replaced by scar tissue.

Carrel and Guthrie tried to transplant adrenal glands maintaining the vascular connections. These attempts were unsuccessful and the tissue necrosed. Better results were obtained by v. Haberer and Stoerck (12) who made autotransplantations into the kidneys of rabbits, dogs and cats. Fifty per cent of their pedunculated grafts took, showing survival of both cortex and medulla. However, only the cortex showed subsequent evidence of regenerative hyperplasia with formation of nodular structures comparable to the common small adenomatous nodules of the adrenal cortex.

Extensive studies were made by Bush, Leonard and Wright (13) on rabbits with grafts in the kidneys, testicles and thyroid gland. Their findings agreed with those of v. Haberer and Stoerck; homotransplants survived up to 247 days. A single attempt with the heterotransplant of a rabbit's adrenal into a patient suffering from Addison's disease met with failure.

A new angle was introduced by Parodi (14) and Neuhauser (15) who used embryonal cortical tissue and obtained not only survival of the graft, but also proliferation of the cortical cells.

While these older experiments left the question as to the specific function of the surviving graft more or less conjectural, the more recent experiments of Jaffe (16) removed all reasonable doubts. Transplanted adrenal cortical tissue not only kept alive animals that had been deprived of their own adrenals but the transplants were also capable of restoring the resistance against infections or intoxications which is strikingly diminished in the adrenalectomized animal. Wyman and tum Suden (17) confirmed the feasibility of both autotransplants and homotransplants, but emphasized that the transplants will not grow in the presence of an adequate amount of the animals' own adrenal tissue.

The cases of suprarenal grafts in humans with Addison's disease can be considered under 3 headings: *1)*, those which were unsuccessful clinically;

2), apparently successful cases in which, however, the fate of the graft could not be determined; *3)*, apparently successful cases in which the fate of the graft could be determined.

In the first group we find the 3 unsuccessful cases reported by v. Eiselsberg. Currie's (18) case of heterotransplantation, although reported as giving a temporary beneficial result, may be classed with the failures. Curschmann (19) used an epileptic's adrenal, but failed to get clinical improvement. D'Abreu (20) reported a case with temporary improvement but death occurred in 19 days. Desmarest and Monier-Vinard's (21) subject died 3 days after transplantation; the gland, which had been transplanted in toto was completely necrotic. Beer and Oppenheimer (22) reported a case in which death occurred 14 days after operation with degeneration in the transplanted cortical tissue.

In the second group belong the cases of Leschke (23) and Reinhart (24) who report on clinically successful transplantation into Addisonian patients of adrenal tissue from patients operated upon for tuberculous kidneys. Beer and Oppenheim reported a second case which survived two separate transplantations for at least 6 months with some clinical improvement. However, the authors felt that since it was still necessary to give the subject a high-salt diet and injections of cortical extracts, the transplant was functionally not completely competent to make up for the adrenal insufficiency. Halpern and Arkusenko (25) reported a case with general improvement for 1 year.

In the third group are two somewhat questionable cases. Kanevskij (26) transplanted a rabbit's adrenal which suppurated and was completely discharged. Clinically, however, the Addisonian patient seemed to be cured, lost his skin pigmentation and showed no return of his former symptoms for 9 months, up to the time of the report. The case of Dimitrijev (27), who transplanted a dog's adrenal under the human skin, is similar. The graft was completely atrophic 10 days after transplantation, yet the patient was clinically improved a month later.

An analysis of these cases suggests the necessity of obtaining some criterion of adrenal cortical activity comparable to the relationship of basal metabolic rate for thyroid function. From a clinical point of view Kanevskij's case of apparent cure with the admission that the transplant did not survive is as valuable as any of the other cases of clinical improvement in which the fate of the transplant was not determined. Since Addisonian patients are known to have periods of spontaneous remission, clinical improvement must be viewed with scepticism. Until some other test of activity is developed, certain knowledge concerning the fate of the transplant is necessary before cure can be claimed. This scepticism must be applied to our case too, but since our patient survived the operation for a considerable period of time without being dependent upon increased salt intake or cortical extracts, both of which were previously indispensable and since, in addition, the transplanted tissue was recovered with morphological evidence of satisfactory preservation, it may at least be said that homo-transplantation of human adrenal cortical tissue can be accomplished with beneficial results.

It might be valuable to inquire into the causes of previous mishaps in the course of adrenal transplantation or into the reason of failures reported by others. It seems that attempts to transplant whole glands or large pieces thereof are doomed to failure, at least in man. A more rational procedure, already suggested by Beer and Oppenheimer, is the grafting of small pieces which have a better chance to become revascularized and thus avoid extensive central necrosis. Several authors believed that the presence of adrenalin in the transplanted tissue is inimical to the procedure and is at least one of the causes of necrosis. While conclusive proof of this point is lacking, it might well be that large amounts of adrenalin in the graft cause vasoconstriction in the operative territory with subsequent anemia and a lessened chance of vascularization. The procedure we followed, *i.e.*, cutting the gland into thin slices and washing them in saline, is likely to remove the major part of the adrenalin and to lessen the intrinsic danger.

One of the main, if not the most important, condition is suitable tissue for grafting. We used hyperplastic gland tissue obtained from a case of cortical hyperfunction. We do not know whether or not this is preferable to the use of normal gland tissue, provided that it is also transplanted without delay after its removal from the donor. Another point, the importance of which remains to be determined, is the fact that both our patient and the donor of the graft belonged to the same blood group, namely, type 2. It is conceivable that incompatibility of the blood is matched by that of other tissues and transplants from incompatible blood groups might prove unsuccessful.

Since the discovery of the cortical hormone and of the value of salt therapy in adrenal insufficiency, the chance of securing favorable results by transplantation is much improved. Patients treated in the accepted way are not only better risks for any surgical procedure, but they would also seem to be better protected against any specific dangers which the sudden introduction of adrenal tissue into the body and the possible necrosis thereof might entail. Administration of cortical extracts after transplantation may also be useful in preventing undesirable strain and exhaustion of the graft which might impair its vitality and its chances of survival. Finally, we wish to emphasize the attempt to stimulate the grafted cortical tissue by injection of the adrenal corticotropic principle. It remains a matter of conjecture whether or not this procedure was really helpful in stimulating the transplant, but it does seem reasonable. The value of this procedure should be further studied both in animals and man. The latter would be more convincing, because the status of a healthy rabbit is hardly comparable to that of an Addisonian patient whose pituitary function might not be entirely adequate.

It remains a puzzling question why our patient with his apparently well-functioning cortical graft developed an attack of weakness after 6 months and finally succumbed to another relapse combined with pneumonia. It may be that while the graft was apparently competent to supply the ordinary amount of hormone for a period of 6 months, a sudden demand for more hormone found it incompetent to meet the emergency. There is evidence to support such a quantitative viewpoint. Thus maintenance of the proper sodium chloride

balance by administration of table-salt reduces the hormone requirements of both the adrenalectomized animal and the Addisonian patient. Smaller doses are required for maintenance and far larger ones to relieve an Addisonian crisis or an acute exacerbation of adrenal insufficiency. Hence the chance to accomplish a cure of Addison's disease would increase with the amount of cortical tissue transplanted. It is also probable that repeated transplants have a better prospect of success than has a single attempt.

SUMMARY

A case of Addison's disease due to atrophy of the adrenal glands is reported. Post-mortem studies showed complete absence of the adrenal cortical tissue. The patient was kept alive by administration of salt and cortical extracts for a period of over 2 years. Interrenin (Goldzieher) seemed to give the best relief.

Adrenal tissue obtained from a case of hypercortical syndrome was transplanted into the rectus muscle. The patient survived for a period of 9 months and appeared to be restored to comparative good health. Autopsy revealed survival of the transplanted cortical tissue in morphologically adequate state of preservation.

The technic and chances of cortical tissue transplantation in Addison's disease are discussed. The use of a donor of the same blood group and stimulation of the graft by injections of the pituitary corticotropic principles are suggested.

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TUBERCULOSIS OF SUPRARENAL GLANDS (ADDISON'S DISEASE)

WITH REPORT OF 7 VERIFIED CASES

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The symptoms of Addison's disease due to fibro-caseous tuberculous lesions are frequently insidious in their onset. Often the nature of the illness is not suspected until years after it origin. It is of interest, therefore, to review the outstanding symptoms in a series of 7 verified cases.

In this group the diagnosis was established by autopsy in 6 cases and by unmistakable x-ray findings in the 7th. The duration of the illness as evidenced by symptoms varied from 2 weeks to 8 years. The average age was 40 years, the youngest being 16 and the oldest 68. Five patients were male and 2 female. All were of the white race. Although negroes constitute a considerable percentage of our hospital admissions, we have never observed Addison's disease in this race.

On autopsy, 5 cases revealed tuberculosis of the suprarenal glands with no tuberculosis elsewhere. Case 2 showed tuberculous involvement of the pituitary gland and in case 6 there was tuberculosis of the vertebrae. The lungs showed no signs of tuberculosis in any of the 7 cases.

There were no family histories to indicate any hereditary tendency. The group included Italian, Norwegian, Polish and American ancestry.

Of especial interest is the incidence of the cardinal symptoms in authenticated cases. Pigmentation was present in 3 of the 7 cases; asthenia in 6; gastro-intestinal symptoms in 6; and abnormally low blood pressure (below 100 mm. systolic) in 4 cases. Three patients had all four of the cardinal symptoms. One patient had none of the cardinal symptoms and no complaints except for generalized pain and cough. Systolic blood pressures ranged from 80 to 186; diastolic, from 55 to 118.

SUMMARY OF CASES

Case 1. A male, age 29, complained of pain over entire body, restlessness and a frequent hacking cough. He was 5 ft. 6 in. tall and weighed 105 lb. Physical examination gave essentially negative findings. There was no history of asthenia, no pigmentation or gastro-intestinal disturbances. Blood pressure was normal. X-ray showed no signs of pulmonary tuberculosis.

At autopsy, diffuse tuberculosis caseation of both suprarenal glands, chronic glomerulonephritis, and edema and congestion of the brain were found. Lung findings were negative.

Case 2. A male, age 45, weight 140 lb., height 5 ft. 5 in., complained of pain in both knees, headaches, dizziness, projectile vomiting, progressive drowsiness and somnolence, and impairment of hearing. The symptoms were of 6 months' duration. Partial blindness of the right eye had been present for 5 months. He appeared anemic. Pupils were fixed to light and accommodation. Hearing of both ears was

impaired. No pigmentation was present. Blood pressure was normal.

At autopsy, both suprarenal glands were found to be enlarged with many areas of tuberculous caseation; the pituitary was greatly enlarged and the seat of diffuse tuberculosis; the brain was edematous and congested; the right kidney was of normal size with cloudy swelling and focal embolic tuberculosis; the lungs were normal.

Case 3. A male, age 28, complained of marked weakness following extraction of 3 upper teeth. He had epigastric distress without pain or nausea for 2 weeks. Six years previously he had noticed that his hands and face were becoming darker than before. Two years previously he had been suspected of having pulmonary tuberculosis. He was 5 ft. 4 in. tall and weighed 125 lb. He appeared languid and apathetic. His teeth were carious. The buccal mucous membrane, face, neck, hands, back, hips, elbows, knees, shoulders and ankles were pigmented to the color of bronze.

His temperature ranged from 99° to 103° F. Blood chemical tests revealed a urea-N of 74 mg., sugar 88 mg., creatinin 1.3 mg. and blood chlorides 300 mg. per 100 cc. of blood. The Wassermann reaction was negative. The red blood cell count was 3,600,000, hemoglobin 70 per cent. The systolic blood pressure ranged from 88 to 106 mm.; the diastolic remained constant at 55 mm.

At autopsy, tuberculosis of both suprarenal glands was found. The left gland was the size of a golf ball with light grayish areas of caseation and several small areas of calcification. The right gland was the size of a walnut with an appearance similar to that of the left. The heart weighed 180 gm. and showed a cloudy swelling. The lungs were normal.

Case 4. A female, age 68, complained of increasing difficulty in urination for 13 months. She became progressively weaker and perspired freely. There was abdominal pain. At 40 years of age she had suffered a paralytic stroke. She had a growth of hair on her chin. There was tenderness in the left lower quadrant of the abdomen; also a stricture of the urethral meatus. There was no pigmentation. The sedimentation time of the erythrocytes was 30 minutes. Occasional pus cells were present in the urine; specific gravity was 1020. Blood pressure was 186/118.

At autopsy, diffuse tuberculosis of both suprarenal glands was found; the heart was slightly enlarged with stenosis of the aortic valves; the spleen was congested with much hemorrhage; the liver showed passive congestion with focal necrosis; in the kidneys there was a large area of cystic degeneration with the entire left renal vein thrombosed and hyalinization of many glomeruli; in the stomach there was diffuse hemorrhagic gastritis; the lungs were sound.

Case 5. A male, age 16, complained of severe pain in the pit of the stomach, continuous vomiting, tired feeling, weakness, dizziness and belching of gas for 5 weeks. His temperature ranged from 99° to 104.5° F. The first test showed the blood pressure to be 90 systolic and 60 diastolic, which pressure gradully decreased to imperceptibility. There was no pigmentation present.

The laboratory findings revealed a basal metabolic rate of +11 to +19 and a blood urea-N of 22 mg. per 100 cc. The red blood cell count was 3,970,000, hemoglobin 60 per cent. The white cell count was 14,700 with 50 per cent polymorphonuclears. X-ray examination showed evidence of bilateral calcification of the suprarenal glands; the lungs were normal. Autopsy was not obtained.

Case 6. A male, age 51, complained of poor appetite and loss of strength of 6 months' duration. Three months after the onset the symptoms became progressively worse. Five weeks before death the patient had frequent vomiting after meals. He had had typhoid fever at 12. During childhood the glands of the neck were swollen. At 36 he had had bronchitis with purulent expectoration. There had been a cold

abscess in the anterior chest wall for 3 years. At 41 he had tuberculosis of the 10th, 11th and 12th dorsal vertebrae and had been bedridden ever since. Pigmentation was present on the hands, neck, axillae, antecubital fossae and buccal surfaces of the mouth. There was clubbing of the fingers. Kyphosis was present in the lower dorsal area. There was impairment of resonance on percussion over lower of both apices. There was a suppurating ulcer to the left of the lower sternum. His temperature ranged from 98.6° to 100.4° F. The blood pressure was 95/70.

Laboratory tests revealed a red blood cell count of 4,800,000, a white cell count of 7,200 with 49 per cent polymorphonuclears and 51 per cent lymphocytes; the hemoglobin was 96 per cent. The blood sugar was 105 mg., urea-N 37.7 mg. and uric acid 2.63 mg. per 100 cc. An x-ray examination revealed kyphosis at the level of the 10th and 11th dorsal vertebrae and a destructive process in the posterior portion of these vertebrae.

At autopsy tuberculosis of both suprarenal glands was found. The left gland showed loss of original outline with many areas of caseation. The right gland was enlarged and a mass of tuberculous caseation. The heart weighed 350 gm. and the left auricle was enlarged. The diaphragm was adherent to the upper surface of the liver, which weighed 1500 gm. There were adhesions about the gallbladder. The spleen weighed 400 gm. and was adherent to surrounding structures. The kidneys showed marked cloudy swelling. The lungs were normal.

Case 7. A female, age 44, complained of fainting spells for 8 years, and blueness of the lips and frequent vomiting for 15 months. She had feelings of exhaustion, headaches, dizziness, poor appetite, thirst and pain in stomach. She had lost 30 lb. in the past 3½ months. She had had an oophorectomy, salpingectomy and hysterectomy performed.

The patient was 5 ft. 1 in. tall and weighed 105 lb.; she appeared apathetic and exhausted. She looked like a mulatto because her skin was very dark and uniformly bronzed, with pigmented spots on her tongue. The mucous membrane of the mouth was dehydrated. Tenderness was present in the epigastrium. Laboratory tests revealed a blood sugar of 80 mg., a urea-N of 48 mg. and chlorides 412.5 mg. per 100 cc. of blood. The urine was normal. The blood pressure was 80 systolic and 60 diastolic.

At autopsy tuberculosis of both suprarenal glands was found. The left gland showed extensive caseation with purulent material throughout the entire lesion and complete obliteration of medulla and cortex, which were twice normal size. The gland was nodular and irregular in outline and disclosed pus pockets just below the surface. The capsule was intact. The right gland was twice normal size and appeared as a caseating cavity with no apparent differentiation between medulla and cortex. The liver was slightly smaller than normal and showed cloudy swelling. The spleen was of normal size. It was congested and contained soft pulp. The heart was normal in size but showed flabby musculature. The lungs were congested with no evidence of tuberculosis.

SUMMARY

A study of 7 verified cases of tuberculosis of the suprarenal glands emphasized the insidious onset of this type of Addison's diseae. All 4 cardinal symptoms were present in 3 cases; none in 1. There was pigmentation in 3 of the 7 cases, asthenia in 6, gastro-intestinal distress in 6 and abnormally low blood pressure in 4. All 7 patients were free from pulmonary tuberculosis. Five had no tuberculous lesions elsewhere, 1 had tuberculosis of the pituitary gland, and 1 tuberculosis of the vertebrae. The duration of the disease as evidenced by symptoms varied from 2 weeks to 8 years.

PUBERTAS PRAECOX (MACROGENITOSOMIA)

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Lisser (1) gives the following description of hypergenitalism in preadolescent males: "The most significant symptoms are premature and excessive development of the genitals, premature change of voice, and rapid excessive development of the body in general. Erections and ejaculations may occur in the first year of life. It is stated that the mental development, though perhaps somewhat precocious, does not keep pace with the generalized precocity; in fact the demeanor of the child may appear to be more childish than is actually the case, because of his nearly adult appearance. The skeleton is influenced in an interesting manner; at first and for several years the boy is large for his age, as if he were becoming a giant, but the excessive output of testicular incretions hastens epiphyseal unification and the premature closure of the latter transposes a misleading and transitory gigantism into a final height which is not excessive and which may indeed incline toward a mild dwarfism. Roentgenograms of such boys will reveal a 'bone age' in advance of their chronological age, as additional proof of precocity."

Although these cases are rare and not often seen in the duration of the average practice the literature is rich in such case reports. Neurath's essay (3), to which reference is frequently made, describes 126 cases of hypergenitalism, 83 females and 43 males. Reuben and Manning (2) reviewed the literature and summarize 398 cases, 243 females and 71 males. Of these 64 came to autopsy or operation.

The pathogenesis of precocious development has been explained in a comparatively small number of this group by tumor formation involving either the pineal, gonads or adrenal cortex. In a few, other hormone abnormalities have been suspected. Perhaps the most complete classification from an etiological view is that recorded by Orel (4). He divides them into 3 groups: *a)*, the primary constitutional group; *b)*, the endocrine group; *c)*, the cerebral group, or cases developing after encephalitis or in association with hydrocephalus or brain tumors.

The primary constitutional group includes those cases in which no incretory or physical abnormality has been discovered to explain the phenomena. Falta (5) remarks that the primary trouble in many cases is apparently in the sexual glandular functions. Zondek (6) speaks of cases in which anomalies are not found and suggests a congenital abnormality derived chiefly from the germinal constitution and causing an abnormal response to hormones which stimulate the sex characteristics. LeMarquand (19) speculates on the possibility of early or late puberty as being a

normal phenomena occurring at an abnormal time. J. Bauer (4) and Orel (4) mention the probability of certain cases being hereditary. Orel (4) reports a case in a male 2½ years old in which some sex abnormalities were found in the family. He also reports the case of 2 brothers who showed pubertas praecox. Stone (quoted by Falta) reports the case of a father and son. Bodd (quoted by Abt, 7) reports the case of a female who started to menstruate regularly at the end of her first year. She was pregnant and delivered an infant at the age of 8. This child also showed marked precocity.

In discussing the endocrine group, a number of glands must be considered. The gonads are the site of a tumor associated with this symptomatology occasionally in girls but rarely in boys. There have been reported about 20 such cases in the female. Four cases have been reported by Sacchi (8), Moreau (9), Rowlands et all (10) and Sutherland (11) with testicular tumors. While in boys a precocious development of the masculine type may be seen, in girls it is possible to have a granulosa tumor develop from the ovary which would give feminine characteristics and also an arrhenoblastoma arising from the ovary which probably has its origin in the rete testis cells; it produces masculinization.

A comparatively small number of cases have been associated with pineal tumors. Laurie (12) speaks of 21 cases of pineal tumors in childhood with autopsy findings. Of these only 8 showed associated pubertas praecox. Horrax and Bailey (13) state that when this condition occurs in a patient with increased intracranial pressure and involvement of the corpra quadrigeminae one must consider a pineal tumor. These cases are found almost entirely in males.

Adrenal cortical tumors are seen most commonly in girls. They are not all associated with precocious sex development. Bulloch and Sequeria (14) collected 25 cases of adrenal tumors. Of these 12 showed associated sex changes and 13 did not. Apert (15) collected 35 cases with autopsy reports. From his study he divided these into 5 types. *A),* Hyperepinephry of the embryonal period; hermaphrodite with internal female organs and outward male appearance. *B),* Hyperepinephry of the fetal period; large clitoris, hypertrichosis. *C),* Hyperepinephry of the prepuberty period; abnormal body development, precocious puberty, adiposity, hypertrichosis and partial hypertrophy of the clitoris. *D),* Hyperepinephry of maturity; menses stop, adiposity develops, hypertrichosis. *E),* Hyperepinephry near and after menopause; marked adiposity and disturbance of sexual functions; sometimes hypertrichosis; symptoms more indefinite.

Krabbe (17) cities 17 cases from the literature, 13 females and 4 males. In the female these all showed virilism but not true precocious development. Lisser (16) speaks of these cases as pseudoprecocious puberty and points out that only one subject reported in the literature menstruated early. The true pubertas praecox in girls as seen in ovarian hypersecretion in early life is characterized by an enlargement of both internal and external genitalia, early menstruation, hypertrophy of breasts and the development of hair (axillary and pubic) of feminine distribution, but not a hypertrichosis. Krabbe (17) suggests that the cortical tumors producing virilism have embryonic testicular cell secretion. Parkes-Weber (10) speaks of two types as seen in childhood, the infant Hercules and the obese, the former being seen only in boys.

A discussion of this kind would hardly be complete without some mention of the gonadotropic hormone of the anterior lobe of the pituitary being considered as causing a premature sex stimulation. Theoretically, at least, it could cause pubertas praecox. Possibly some of the so-called primary hypergenitalism cases might be explained by this mechanism. Formerly there has not been any method by which

this activity could be measured but now prolan estimations can be made. A number of text books Zondek (6), Falta (5) and Goldzieher (23) refer to the pituitary as a possible cause. Laurie (12), however, states that there has never been a reported case of a pituitary lesion producing this syndrome although the reverse is frequently seen. As it is known that a balance is maintained between the pituitary sex-hormones and the gonad sex-hormones, it would be expected that the overacting gonad would cause a cessation of secretion of gonadotropic hormone. This is seen experimentally when estrogenic substance is administered and Moore (18) reports that in the male the effect of gonad hormone inhibits the pituitary with a resultant failure of testis function. Prolan estimations in these cases can be made in the future and will help in settling this question.

Before leaving the endocrine etiology it might be noted that Asher has obtained a thymic extract which is reported to be a powerful sex stimulant. Other authors have reported sex gland atrophy following thymic feeding. There are no reported cases in the literature of the thymus causing this syndrome.

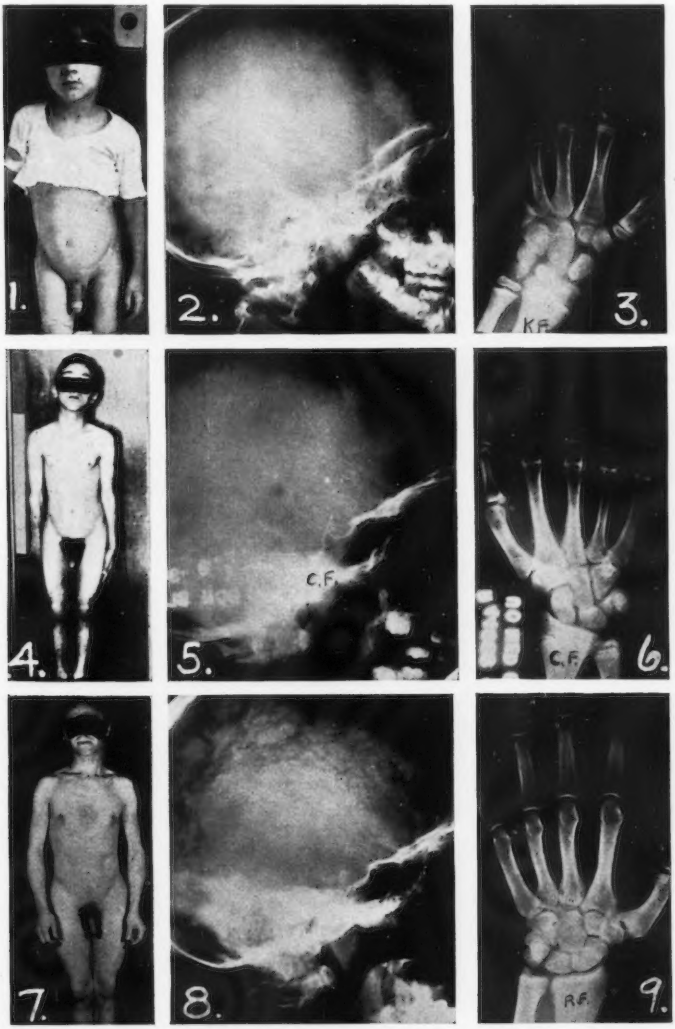
A few cases have been reported in which mid-brain lesions with no evidence of endocrine abnormalities produced this syndrome. LaMarquand (12) reports a most complete study of such a case. In his case it appeared as though a redundant portion of brain had been formed by developmental error which had not produced degenerative changes by pressure in the hypothalamic area but which was connected by tracts to it. He also states that Henyer reports a case of pubertas praecox in which a similar tumor was found. Other cases reported by Horrex and Bailey (13) and Dods (20) were associated with mid-brain tumors. On the other hand Thomas and Shaeffer (21) and Von Economo (22) mention inflammatory lesions in the mid-brain associated with this syndrome.

We have had an opportunity of observing an unusual family in which all male members, the father and both sons, developed precociously. The younger son, age 5, began his unusual development at about the age of 3. The older son, age 8, first showed signs of precocity when 18 months old. The father, age 33, believes that he began his rapid development at the age of 5. It is further interesting to note that the paternal grandfather and great grandfather also have a history of this same type of development. There were also 2 great uncles and 1 distant cousin (male) who have the same history. This makes 8 known instances in 4 generations in which pubertas praecox was present. These instances are all in males and have all been transmitted through males with one exception, that being the distant cousin. There have been known no instances in the family of precocious development in the females.

K. F., Age 5, Male. This patient was first seen by Dr. Bilderback at the age of 4. At that time he was admitted to the Doernbecker Hospital because of a bronchopneumonia. It was noted at that time that he was large for his age, had a deep voice and that the external genitalia were markedly developed with a beginning growth of pubic hair. However, the child was taken from the hospital before complete studies could be made. He re-entered the hospital this past winter because of an infected hand. At that time an opportunity was presented for a more careful consideration of the precocious development.

He had a normal birth, weighing 4.5 lb. The parents stated that he seemed to develop normally. He cut his first teeth at 6 months, walked at 14 months; nothing unusual was noted as to his time of talking. When he was about 3 years of age it

was noted that he began to grow more rapidly and the genitalia began to develop. At about 3.5 to 4 years pubic hair began to grow and during the past 6 months a



1. Case K. F., age 4 yrs. *2.* Lateral skull roentgenogram. *3.* Showing advanced bone age of about 12 yr.; chronological age 5 yr.
4. Case C. F., age 8 yr. *5.* Lateral view of skull. *6.* Showing advanced bone age of about 17 yr.; chronological age 8 yr.
7. Case R. F., age 33 yr. *8.* Showing lateral view of skull, calcified pineal and diffuse atrophy of skull bones. *9.* Showing adult normal wrist bones.

moustache had become noticeable so that the mother shaved him every few days to prevent childhood teasing. The voice, which had always been deep in pitch, seems more so now and the pitch changes with speaking, as might be expected in a lad

of 14. There has not been any particular attraction to girls. There have been no known emissions. The mental development seems about average for a child of 5 and his interests are in toys and games.

His past history was not unusual. He had had enuresis which has not completely cleared at the present time.

The physical findings were essentially normal, except for the following points. The body measurements were about those of a normal lad of 8 years. A definite moustasche of dark hair was seen but this was not profuse. There was no axillary hair present. The pubic hair was present but scant. The penis, scrotum and testis were fully developed. The prostate was palpable and well formed. A careful neurological examination was made. The eye movements, visual fields and eye grounds were normal. There was no disturbance of sensation, muscle sense or in reflexes.

The laboratory tests revealed a B.M.R. of +3, normal urinary constituents, and a blood count showing: hemoglobin, 112; R.B.C. 5,680,000; W.B.C. 11,300 (infected hand); polymorphonuclear neutrophiles 62 per cent; basophiles 1 per cent; small lymphocytes 31 per cent; monocytes 2 per cent; staff cells 2 per cent. The blood constituents were: urea nitrogen 18.5 mg., calcium 14 mg., phosphorus 5.6 mg., sugar 93 mg., and chlorides 396 mg. per cent. The glucose tolerance test revealed a fasting blood sugar of 87 mg. One-half hour after the sugar meal the blood sugar rose to 148 mg., 1 hr. 143 mg., 1.5 hr. 96 mg. and 2 hr. 67 mg. per cent. The spinal fluid was not under abnormal pressure, it was clear and the cell count was 8, the total protein was 46.3 mg., the globulin 1 plus, the Wassermann reaction negative and the Lange negative. The urine was extracted to obtain the prolan content. This was tested on immature female white rats and the reaction was negative. The urine was also extracted to obtain male sex hormone; this extract was tested upon the capon for comb growth and the reaction was positive. A lateral skull plate showed a normal sella and no evidence of increased intracranial pressure. A flat plate of the chest (P.A.) showed normal heart, aortic and lung shadows. There was no unusual mediastinal shadow. The plates following intravenous pyelograms showed no change in the size or position of either kidney. Wrists and hands showed an increased development of all bones and suggested a bone age of about 12 years.

C. F., Age 8, Male. This patient, a brother of preceding subject, was first admitted to the hospital May 4, 1934, because of joint pain. Hypertrophic infected tonsils were found and tonsillectomy advised, for which he was brought into the hospital 1 month later. Examination revealed an enlarged thyroid, a systolic murmur present over the whole precordium and precocious development, the child being large for his age, having a deep voice and the external sex organs being remarkably developed with abundant pubic hair. The tonsillectomy was performed and the child was discharged. He re-entered the hospital on December 28, 1935, because of a bilateral otitis media and at this time we had an opportunity of further study.

The past history was as follows. At about the age of 18 months it was noted that his external genitalia began to develop rapidly and pubic hair began to grow. At 2 years of age his general body growth seemed accelerated. At 6 years, pubic hair was abundant, axillary hair was present, and the beard began to grow necessitating shaving. About this time, or perhaps a little later, the parents noted that he was sex-conscious although up to the present time he has not been attracted to the opposite sex. Masturbation had been practiced and the father stated that emissions occurred. His mental development was about average for his chronological age. Aside from the complaints mentioned above, his past history was not unusual except that he had frequent colds and earaches.

Physical examination revealed a lad who appeared about the size of a 16 year-old boy. He was rather pale and undernourished, the result of his recent infection. The head and neck were normal except for the otitis media and a moderate cervical lymphadenitis. The thyroid was slightly enlarged. The chest was not unusual, the lungs being clear. The heart seemed somewhat enlarged to the left with a diffuse P.M.I. A systolic murmur was heard over the precordium with greatest intensity over the apex. The abdomen was normal, as were the extremities. The external genitalia were completely developed. The pubic hair was of masculine distribution. Axillary hair was present. A moustache was seen but the rest of the beard was barely visible. Mild acne was present on the face. The prostate and seminal vesicles were both palpable and well formed.

There were no abnormal neurological findings. The eye grounds and visual fields were unchanged. Eye movements were normal. Cutaneous sensation, muscle sense and reflexes all seemed normal. The body measurements were about those of a normal 15 or 16 years old boy except for weight.

The following laboratory results were obtained. The urine constituents were essentially normal although a few calcium oxalate crystals were seen. The blood count showed: hemoglobin 100; R.B.C. 5,180,000; W.B.C. 11,400 (ear infection); polymorphonuclear neutrophiles 77 per cent; eosinophiles 2 per cent, small lymphocytes 13 per cent; monocytes 2 per cent; staff cells 5 per cent. The blood constituents were: calcium 12 mg., phosphorus 4.2 mg., and chlorides 495 mg. per cent. The glucose tolerance test was as follows: the fasting blood sugar was 82 mg., ½ hr. after a glucose meal the blood sugar rose to 151 mg., in 1 hr. it was 154 mg. and at 2 hr. 106 mg. Due to his fever while in the hospital no B.M.R. was determined. The tuberculin test gave negative findings. The urine was extracted to obtain the prolan content. This was tested on immature female white rats and the result was negative. The urine was also extracted to obtain male sex hormone; this extract was tested upon the capon for comb growth and the result was positive. A lateral skull plate showed a normal sella and no evidence of increased intracranial pressure. The sphenoid sinus, however, was very large. The renal shadows were definitely seen and appeared to be of normal size, shape and position. A flat plate of the chest (P.A.) showed some increase in hilus markings. The cardiovascular shadows seemed about normal. There was no evidence of an unusual upper mediastinal shadow. The hands and wrists showed an increased development of all bones and suggested a bone age of about 17 years.

R. F., Age 33, Male. This man, father of the 2 preceding subjects, presented himself, at our request, in Jan. 1936 for an endocrine study. He stated that his health was excellent. He was born in Indiana, came to the west coast at about 4 years of age and had lived in this part of the country ever since. He believes he was somewhat slow in starting to walk and talk but thinks he cut his teeth at an early age. He states that his sex development seemed complete at about the age of 5 and, when he was 7 or 8, he had the sexual urge and desires of a mature lad. He was attracted to the opposite sex at this time. He further states that for the past few years, although he is only 33 years of age now, he has not had the sex urge which he formerly had although he attributes this lack to being interested in other things. He further states that as a lad his voice was very low pitched and he used to sing bass but for the past 5 or 6 years his voice has become higher pitched and he now sings tenor. He also states that he grew fast and was larger than the rest of his playmates. This growth seemed to be present until he was about 14 years of age at which time he stopped growing so that now his stature is somewhat less than average.

He had typhoid fever at about the age of 6 years. He also had had sinus trouble. He teeth had deteriorated rapidly and seem soft, necessitating considerable dental work. He stated that he passes an increased amount of urine, particularly if excited or during cold weather. However, a 24 hour sample, which was saved, was not unusual in quantity. In 1918 he had a severe attack of influenza and following this had been bothered somewhat with indigestion.

This patient appeared to be of normal proportions but of small stature. The head and neck were essentially normal except for the presence of poor teeth. The chest was not unusual and the lungs and heart seemed normal. The abdomen was essentially normal as were the extremities. The external genitalia were well developed although the left testis was only about half the size of the right. The hair distribution was typically masculine. The pubic and axillary hair seemed normal as did the beard. There was a slight amount of hair present on the chest. The rectal examination revealed a normal, palpable prostate and seminal vesicles. The body measurements are shown in the table.

The laboratory findings were as follows. The B.M.R. was —1. The urinary constituents were not unusual. The blood count showed: hemoglobin 88; R.B.C. 4,540,000; W.B.C. 6,000; polymorphonuclear neutrophiles 66 per cent; lymphocytes 32 per cent; monocytes 2 per cent. The blood constituents were: urea nitrogen 13 mg., calcium 11.6 mg., and phosphorus 2.4 mg. per cent. The glucose tolerance test revealed a fasting blood sugar of 82 mg.; 1 hr. after the glucose meal the blood sugar was 167 mg., and 2 hr. afterward 104 mg. per cent. The blood Wassermann reaction was negative. The urine was extracted to obtain the prolan content. This was tested on immature female white rats and the result was negative. The urine was also extracted to obtain male sex hormone; this extract was tested upon the capon for comb growth and the reaction was positive. There was a diffuse atrophy involving all the bones of the skull. There was no evidence of change in the sella or increase in the intracranial pressure. A pineal shadow was evident. A flat plate of the chest (P.A.) revealed no abnormal shadows except some increase in hilus markings. There was no evidence of enlargement or change in the position of either kidney on a K.U.B. The wrists and hands showed normal adult bones.

	K.F.	C.F.	R.F.
Height, inches	49.5	66.5	64
Weight, pounds	51	88	115
Trunk, inches	25.5	31.5	31
Lower extremities	24	35	33
Span, inches	53.5	62	66

The varied pathological processes which seem capable of producing pubertas praecox led LeMarquand to advance the following hypothesis: "In cases in which the pineal, the adrenal, the hypothalamus, or the gonads are involved, they each and all cause pubertas praecox by an influence on the hypothalamic centers and from thence on the pituitary." In our cases we were unable to demonstrate any increased maturity function on the part of the pituitary. As has been mentioned previously, one must bear in mind that the possibility of a hereditary factor causing the germinal cells to be stimulated by less than a normal amount of hormone may be the explanation in these constitutional cases.

SUMMARY

Three cases of typical hypergenitalism (macrogenitosomia) occurring in one family are reported. Male sex hormone was demonstrated in the urine of

each of the 3 by use of the capon-comb test. Normally this is not found in boys under 10 years of age. Prolan could not be demonstrated by the action of urine-extract in immature female rats in any of the cases. No associated signs or symptoms were present from which to suspect tumor or marked hypertrophy of the pineal, adrenal cortex, gonads, pituitary or thymus. There were no signs or symptoms from which a cerebral lesion might be suspected. These cases seem to fall in the group called 'primary, primary-constitutional, or congenital pubertas praecox.'

In addition to the 3 cases reported the family history disclosed 5 other instances in 4 generations. The condition seems, therefore, to have been genetically determined. It is possible that what was inherited may have been an abnormal responsivity to gonadotropic hormones.

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CLINICAL AND LABORATORY NOTES

A SHORT TEST FOR OVARIAN FOLLICULAR HORMONE AND OTHER ESTROGENS

EDGAR ALLEN, GEORGE M. SMITH AND W. U. GARDNER*, †

The test in general use for assay and standardization of estrogenic substances involves stimulation by the injected hormone of full estrous growth of the vaginal epithelium in the ovariectomized rat or mouse. This requires at least 48 to 56 hours, sometimes longer, but as followed by the smear method it provides a definite, clear-cut endpoint.[1, 2] The modification here proposed makes a saving in time without sacrificing the clear-cut nature of the endpoint.

Following reports that colchicine arrests dividing cells in mitosis,[3, 4, 5, 6] we reported that the use of colchicine accentuates the growth effect of theelin (estrin) on genital tissues by arresting dividing cells in mitosis so that they can be accumulated for definite periods of growth.[7] Growth (mitotic division) in the vagina, uterus and mammary glands was stimulated by theelin†† and the evidence gathered by holding the dividing cells in metaphase (colchicine effect). In this condition the cells stand out so clearly in the sections that they are easily recognized. As early as 9½ hours after injections of theelin and colchicine, enough cells had been held in mitosis so that sections of the vaginal wall showed this response. At 13½ hours after theelin was injected the effect was more widespread. This effect may be used as an endpoint in a short test for estrogenic substances.

The procedure in the proposed short test follows. *1)*. Young adult rats or mice are ovariectomized and castrate atrophy allowed to continue for 10 days. *2)*. The material to be tested or standardized (the estrogen) and the colchicine (0.1 mg. in acqueous solution) are *a)*, injected subcutaneously late in the evening or *b)*, the estrogen late in the afternoon and the colchicine late in the evening. *3)*. A biopsy specimen of vagina is removed the next morning approximately 9½ hours after the injection of colchicine.§ This is taken from the lateral vaginal wall by clipping off a piece grasped by toothed forceps. *4)*. This specimen is sectioned by the freezing technique, stained lightly and mounted. *5)*. Identification of frequent mitoses in the basal layers of the epithelium is the criterion of a positive test.

There are several drawbacks to this proposed short test. In the first place, it is not as easy as the vaginal smear test because it requires more careful p.eparation of the specimen. Secondly, the growth in the vagina may not be evenly distributed.

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† This work is being supported by grants from the Fluid Research Fund and the Atypical Growth Fund of Yale University School of Medicine.

†† The theelin used in these experiments was supplied by Parke, Davis and Co.

§ The optimum time for the colchincine effect in the mouse was found by Dustin (1935) to be 9½ hours.

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Frequently mitoses are found in the lateral walls of the vagina before they appear in the mid-line regions and sometimes on one side before the other. For this reason and because there is also less danger of damage to urethra and rectum, the biopsy specimens should be taken from the lateral walls. Thirdly, if the atrophic vaginal wall is heavily infiltrated with leucocytes, care must be taken to differentiate them from arrested mitoses in which the chromatin material may be clumped.

This short test for estrogenic substances is truly a measurement of growth by cell multiplication (mitosis, hyperplasia) as distinct from other factors, such as increase in size of cells, blood supply, tissue fluids, or accumulated secretions, any or all of which may contribute to increase in size or weight—the criterion often used to determine responses of organs to hormones.

Summary. A short test for estrogenic substances is described; it may be completed in 10 to 16 hours as compared with 48 to 56 hours for the vaginal smear test. It substitutes recognition of early growth produced by the estrogenic hormone in the vaginal wall for the complete estrous growth as required by the original test. This is made possible without sacrificing the clear-cut nature of the endpoint, by use of colchicine with the estrogenic material to be tested. Active estrogenic material starts mitotic division of the basal cells of the vaginal epithelium. Colchicine arrests the mitoses, thus accumulating the evidence of growth. Biopsy specimens of vagina are used instead of vaginal smears for determination of the endpoint.

ESTROGENIC HORMONES IN THE OVARIES OF SWORDFISH

ABNER I. WEISMAN, DANIEL I. MISHKIND, ISRAEL S. KLEINER AND CHRISTOPHER W. COATES*

In a previous paper[1] it was shown that upon extracting the ovaries of 30 mature herring in the resting phase of the ovulatory cycle, no estrogenic hormones could be found, using immature female mice as the test animals. It was concluded, therefore, that fish ovaries either do not contain the estrogenic hormones or that the hormones are not present in large enough amounts to produce the estrus reaction. A much larger quantity of fish ovaries was later obtained. Freshly castrated rats were used as the test animals, and the extraction of the hormones was performed with a more refined technique[2] which resulted in more efficient extraction.

Experimental. Three pairs of swordfish ovaries (*Xiphias gladius*)§ weighing 0.9, 1.7, and 2.1 kg. per pair, respectively, were used. These ovaries were obtained through the courtesy of Miss F. LaMonte, Associate Curator of Ichthyology, American Museum of Natural History, who collected them on the Lerner-Cape Breton Expedition in the summer of 1936. In all, the 3 pairs of ovaries totaled approximately 10 lb. in weight, about 25 times the weight of the 30 pairs of herring ovaries used in the previous experiment. The swordfish ovaries were cut into strips, finely ground, and extracted with 2 liters of chloroform after acidification with concentrated HCl. The mixture was refluxed for 8 hours, and the chloroform extract evaporated to dryness. The gummy residue was then dissolved in ether and shaken with 2N KOH until no further color appeared in the aqueous phase. The washings were extracted with ether, the ether solutions combined, and evaporated

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§ We wish to acknowledge the assistance of Dr. F. D. Speer for the histological sections of the swordfish ovaries. We are also grateful to Drs. R. F. Nigrelli and R. G. Janes of the New York Aquarium and the New York University for definitely identifying the swordfish gonads as ovarian.

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to dryness. The residue was refluxed with 50 cc. of 3N KOH in methyl alcohol for 2 hours. After cooling, 2 liters of water was added and the mixture was extracted repeatedly with ether. The aqueous fraction was saved. The extract was washed with water, dilute acid, and finally again with water. This constitutes the male hormone fraction. The aqueous fraction was treated with HCl until acid to Congo-red and an additional 20 cc. of conc. HCl was added. The mixture was heated on the steam bath for 1 hour and then extracted repeatedly with ether. The ether extract was washed with water, dilute alkali, and finally with water. This yielded the female hormone fraction and amounted to 17 cc. of an oily viscous substance. It solidified at room temperature and slight warming rendered it liquid. The male fraction was a solid of a waxy consistency, weighing 5.2 gm. It was necessary to dissolve this in 50 cc. of cotton-seed oil so that it could be injected.

Procedure. Immature female mice were first used as the test animals but the first injection of the extracts proved toxic to the mice. It was then considered advisable to use a larger animal and accordingly, 6 mature female rats were freshly castrated through a dorsal incision[3] and were ready for use 10 days after operation. The rats were divided into 2 groups of 3 rats each. Each animal in group A was injected with 1 cc. of the original 17 cc. of female hormone fraction daily for 3 days. Each animal in group B was injected with the male hormone fraction and received the total equivalent of 0.5 gm. of the original waxy substance in cotton seed oil in the course of 3 days. Vaginal smears were taken daily up to 144 hours.

Results. After 96 hours and up to 144 hours the fraction supposedly containing the male hormone produced no estrus in the castrated female rats. This served as our control experiment. The results from the female hormone fraction were surprising in that 2 of the 3 rats showed definite estrus reaction at 96 hours. One rat gave the typical epithelial desquamation showing only the hyaline-like, desquamated, non-nucleated epithelial cells characteristic of true estrus. The second animal showed a positive reaction but even at 144 hours, the hyaline cells were outnumbered by nucleated cuboidal cells. This might be called a 'threshold reaction'[4] which is positive but shows evidence of being less than 1 R.U. There were no leucocytes present in either vaginal smear. The third animal developed toxemia and died at or before 72 hours with the autopsy revealing only slight congestion of the tubes and only slight enlargement of the uterus.

Discussion. These results do not conflict with the previous paper[1] in which it was stated that no estrogenic hormones could be found in herring ovaries, since too small an amount of tissue was used in the earlier experiment. Even in 10 lb. of swordfish ovaries only a small amount of estrogenic hormone was discovered. Since only 1 rat gave a 4 plus reaction, although the other 2 also showed some signs of estrus, it is evident that the amount of substance injected into each animal was 1 R.U. or less of estrogenic hormone. Since the total amount of extract was 17 cc. and since 3 cc. of the extract produced a reaction typical of not more than 1 R.U. the 10 lb. of swordfish ovaries contained less than 6 R.U. of estrogenic hormone. It can be readily seen why no estrogenic reaction was obtained in the earlier experiments since, in all probability, the 30 pairs of herring ovaries weighing less than half a pound perhaps did not contain 1 R.U. of this substance altogether.

Summary. Less than 6 R.U. of estrogenic hormone could be extracted from 10 lb. of swordfish ovaries as shown by the estrus reaction on freshly castrated rats.

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ASSOCIATION NOTICE

SCIENTIFIC PROGRAM OF THE TWENTY-FIRST ANNUAL MEETING

Haddon Hall, Atlantic City, N.J., June 7 and 8, 1937

The Neural Basis of Estrual Behavior in the Cat. Philip Bard, *Baltimore, Md.*

Pituitary Dwarfism with Diabetes Mellitus: Response to Treatment. Harvey G. Beck and George M. Suter, *Baltimore, Md.*

Further Studies on Anterior Lobe Hormones. J. B. Collip, *Montreal, Que.*

Cushing's Disease. Charles William Dunn, *Philadelphia, Pa.*

Search for a Diabetogenic Hormone. Joseph Eidelsberg, *New York City.*

Histological and Cytological Changes in the Endometrium of the Monkey, in Relation to Hormone Balance. Earl T. Engle and A. E. Severinghaus, *New York City.*

The Diversified Clinical and Pathological Pictures of Failing Ovarian Function. Rita S. Finkler, *Newark, N.J.*

The Influence of the Adrenal Cortical Hormone and Allied Compounds upon Salt and Water Excretion. George A. Harrop, *Baltimore, Md.*

Certain Aspects of the Adrenal Cortex in Relation to Carbohydrates. Frank A. Hartman, *Columbus, Ohio.*

Gonadotropic and Gonadotropic-Like Factors in the Treatment of Sterility in the Male. N. J. Heckel, *Chicago, Ill.*

Effects of Insulin on Cell Proliferation. R. G. Hoskins, *Boston, Mass.*

Does a Difference in Effect on the Rat Exist between Crude Emmenin, Estriol Glycuronate, and Estriol? A. C. Ivy and R. R. Greene, *Chicago, Ill.*

Interrelationships between Vitamins and Endocrines. C. G. King, *Pittsburgh, Pa.*

The Daily Excretion of Androgenic and Estrogenic Substances in Normal and Diseased Conditions in Men. F. C. Koch, A. T. Kenyon, T. F. Gallagher and D. H. Peterson, *Chicago, Ill.*

The Action of Pituitrin, Estradiol and Progesterone on the Human Uterus in Vivo. Raphael Kurzrok, Hans Wiesbader, Michael M. Mulinos and Benjamin P. Watson, *New York City.*

The Mechanism of the "Diabetogenic" Action of the Pituitary. C. N. H. Long, *New Haven, Conn.*

Familial Intersexuality—A Report of Three Unusual Cases. Daniel R. Mishell, *New York City.*

Analysis of Amenorrhoea by Prolactin and the Luteinizing Hormone. Ira T. Nathanson and Harry L. Fevold, *Boston, Mass.*

Atypical Uterine Growths Produced by the Prolonged Administration of Oestrogenic Hormones. Warren O. Nelson, *Detroit, Mich.*

Thyroid Instability, its Detection, Measurement and Treatment. E. Obermer, *London, England.*

President's Address. F. M. Pottenger, *Los Angeles, Calif.*

Male Sex-Stimulating and Female Sex-Repressing Hormone from the Adrenal. F. M. Pottenger, Jr., and D. G. Simonsen, *Monrovia, Calif.*

Glandular Control of Appetite. Curt P. Richter, *Baltimore, Md.*

Some Endocrine Aspects of Growth. Oscar Riddle, Robert W. Bates, James P. Schooley, Ernest L. Lahr, and Mychyle W. Johnson, *Cold Spring Harbor, Long Island, N.Y.*

Responses of the Human Ovary to Gonadotropic Principles. R. A. Ross and E. C. Hamblen, *Durham, N.C.*

The Rôle in Growth and Development of Certain Potent Chemical Agents found in Thymus Extract. Leonard G. Rowntree, Arthur Steinberg, N. H. Einhorn, N. K. Schaffer and William Ziegler, *Philadelphia, Pa.*

The Oral Administration of Hormones. William T. Salter and Hildegarde Wilson, *Boston, Mass.*

The Use of Adrenal Cortex Preparations in the Treatment of Asthenias. Elmer T. Sevringhaus, *Madison, Wis.*

Studies in Creatinuria. E. Kost Shelton, and B. N. Tager, *Los Angeles, Calif.*

The Effect of the Female Sex Hormones on Immunity and Allergy. Douglas H. Sprunt, *Durham, N.C.*

Further Observations on the Treatment of Hypogenitalism and Undescended Testes, with Special Reference to the Production of Premature Puberty. Willard Owen Thompson, *Chicago, Ill.*

A Study of the Mechanism of Edema Associated with Menstruation. George W. Thorn, *Baltimore, Md.*

PAPERS TO BE READ BY TITLE

The Clinical Value of Hormone Determination in Endocrine Disorders. A. S. Blumgarten, *New York City.*

Clinical Studies of the Function of the Corpus Luteum in some Abnormal Conditions of the Menstrual Cycle and in Pregnancy. J. S. L. Browne, J. S. Henry and Eleanor M. Venning, *Montreal, Que.*

The Hormone Content of the Urine of Children. Ralph I. Dorfman and William W. Greulich, *New Haven, Conn.*

Female Pseudo-Hermaphroditism in Siblings with Adrenal Hyperplasia and with Evidences of Atypical Precocious Maturity. Theodore O. Elterich, *Pittsburgh, Pa.*

Preliminary Report on the Possibility of the Existence of an Endometrial Hormone. George Joyce Hall, *Sacramento, Calif.*

The Effect of Hypoglycemia on the Metabolism of the Brain. Harold E. Himwich, and J. F. Fazekas, *Albany, N.Y.*

A Report on the Treatment of Essential Hypertension and Diabetes Mellitus by Irradiation of the Pituitary and Adrenal Regions. James H. Hutton, *Chicago, Ill.*

Gonadotropic Effect of Various Urinary Extracts in the Male Rat. J. A. Morrell, *New Brunswick, N.J.*

Ovulation and the Oxygen Consumption of Rabbit Anterior Pituitary. Boris B. Rubenstein, *Cleveland, Ohio.*

The Thyrotropic Hormone in Relation to Exophthalmic Goiter and Toxic Adenoma. August A. Werner, *St. Louis, Mo.*

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

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ADVANCE ABSTRACTS

The excretion of gonadotropin by normal human males after the ingestion and injection of extracts of pregnancy urine. Friedman, M. H., and G. L. Weinstein. To appear in this Journal.

Benzoic acid (Katzman-Doisy) preparations of 24-hour urine samples from normal males revealed a daily excretion of about 6 RB.U. of gonadotropic material. Tannic acid (Levin) determinations on specimens from the same individuals gave consistently higher yields. Neither the oral ingestion of 8,000 to 42,000, nor the intramuscular injection of 480 rat units of P.U. had any detectable influence on the excretion of gonadotropin. The intramuscular injection of 600 to 750 R.U. of P.U. led to an increased excretion of varying degree, amounting to 20% or less of the injected activity. During the course of the injections, 1 individual, with a history of allergy, developed hypersensitivity to the P.U. extract as evidenced by a positive skin reaction, and by an alarming general reaction to an intramuscular injection of P.U. which was made several months after the preceding injection. However, the intramuscular injection of 500 to 750 R.U. of P.U. into the other subjects did not evoke severe systemic reactions.

The specificity of progesterone in inducing sexual receptivity in the ovariectomized guinea pig. Hertz, Roy, R. K. Meyer and M. A. Spielman. To appear in this Journal.

Seven compounds closely related to progesterone (pregnandione, pregnandiol, luteosterone-A, estriol, androstendione, dehydroandrosterone, and testosterone) proved unable to produce the copulatory reflex in the theelin conditioned, ovariectomized guinea pig. Synthetic crystalline progesterone and oestrin-free, sow corpus luteum extracts in adequate dosage proved uniformly effective. Assays of lower doses of progesterone using sexual receptivity in the guinea pig as an end point can detect as little as 0.05 I.U. of progesterone. The method is consequently offered as a sensitive and specific assay for progesterone.

Accentuation of the growth effect of prolactin on the crop gland of the pigeon by arrest of mitoses with colchicine. Leblond, C. P., and E. Allen. To appear in this Journal.

The development of the pigeon's crop gland induced by prolactin may be accentuated by the use of colchicine. Following injections of this alkaloid, mitoses are held and accumulated at the metaphase stage, their number being maximal after 10 hours. Sections of the growing crop gland of animals treated with prolactin alone show less than 1% of the cells in mitosis. Resting crop glands, of control animals given colchicine alone, contain ½ to 2% of the cells arrested in mitosis. In a pigeon treated with prolactin and colchicine, from 10 to 53% of the cells are dividing. Such a number of mitoses would be a true picture of the number of cells which would have completed division during the time of action of colchicine (10 hours). After colchicine, the ratio of dividing nuclei to the total number of nuclei (mitotic index) might be used as a test of the action of prolactin. Colchicine has also been successfully used to estimate the crop gland growth induced by local injections of prolactin or implants of pituitary tissue under the skin of the crop gland.

Clinical experiences with protamine insulin in its various modifications. Rudy, A. To appear in this Journal.

The effect of protamine insulin in its various modifications has been studied in over 100 cases of diabetes mellitus. Several methods of treatment have been tried in long intervals in patients who have been under close supervision for a number of years previous to these studies. The records of 4 cases are presented in detail. With protamine-zinc-insulin many uncomplicated cases of diabetes are best regulated on a single morning dose. In diabetic coma and in other complications protamine-zinc-insulin should be used only to supplement regular insulin. The diet plays an important role in the treatment. The carbohydrate of the diet should be divided so as to avoid insulin reactions, which at times can be quite severe. Reactions can occur at any time of the day or night. Individualization of every case is necessary.

Reversal of the estrin-induced prostatic pathology in mice by the use of testosterone. Rusch, H. P. To appear in this Journal.

Long-period injections of estrin into male mice induce changes in the prostate gland characterized by a metaplasia of the glandular epithelium into a stratified squamous type, an increase in the fibromuscular stroma, and a weight decrease of the whole gland. Testosterone, when administered simultaneously with estrin, after these pathological changes had been established, induced a reversal of the metaplasia toward a normal type and was responsible for a weight increase of the gland, but had little effect upon the fibromuscular stroma. Progesterone and dehydroandrosterone, in the doses used, had no such effect.

Effect of emmenin on gonadotropic hormone excretion in castrate and spontaneous menopause. Salmon, U. J., and R. T. Frank. To appear in this Journal.

Two surgical castrates and 1 spontaneous menopause, all of whom showed increased excretion of gonadotropic hormone in the urine, were treated with large doses of Collip's emmenin. Diminution or disappearance of the gonadotropic hormones from the urine were noted after the treatment had been continued for some time. Simultaneously the vaginal spread changed from the negative or pseudo-positive to positive. Coincidentally the neurovascular symptoms diminished or disappeared. The conclusion is drawn that emmenin, presumably an estriol glucuronide, in sufficient dosage, acts similarly to the other estrogenic substances, by inhibiting the excessive action of the prepituitary.

Male hormone and the testis-comb relationship in the chick. Breneman, W. R. To appear in this Journal.

Dihydroandrosterone-benzoate (Oreton B) and testosterone (Oreton) injected for 5 days into 5 or 10 day old chicks caused immediate cessation of testicular growth which apparently lasted only during the period of injection. Chicks injected for the same 5-day period but not killed until 30 days old, had an average gonad weight 90% higher than the controls. This was in harmony with the augmented testicular response observed when pituitary hormone was given for 5 days following 5 days of Oreton B-treatment. Combination injection of pituitary extract with Oreton B showed that the latter was acting indirectly on the gonads by a suppression of secretion or release of hormone from the animals' own pituitary. The ratio of comb growth in Oreton B-treated chicks to that of the controls at 10 days was 1.8 to 1, and at 30 days the ratio was 1.9 to 1. The amount of comb growth tended to be correlated with gonad weight. The possibility that difference in the rate of male hormone secretion due to differential development of the seminiferous tubules was advanced as an explanation for the exceptions which were noted. A total of 270 chicks were used for this work.

The use of glycerol extract of the adrenal cortex in the treatment of adrenal insufficiency. Hartman, F. A., G. W. Thorn and R. R. Durant. To appear in this Journal.

The use of a glycerol extract of the adrenal cortex, from which most of the epinephrin has been removed, is described. This extract contains cortin because it can substitute for one-third to one-half of the cortin requirement in adrenalectomized cats. Three cases of severe Addison's disease treated with this extract are described in detail to show the limitation of the therapy. Patients with less severe symptoms may respond so well that by continued treatment they return to normal activity. The lower cost of glycerol extract makes continued treatment feasible.

Ovaries secrete male hormone. I. Restoration of the castrate type of seminal vesicle and prostate glands to normal by grafts of ovaries in mice. Hill, R. T. To appear in this Journal.

It has been found that ovaries grafted into the ears of castrate male mice secrete male hormone. The criterion used for the presence of male hormone is the response obtained on the accessory sexual glands of the castrated host. Thirteen animals, bearing grafts of ovaries in their ears for 92-301 days were killed. Normal seminal vesicles and prostates were found in 6 animals; partially developed glands in 2, and the remaining 5 were of a complete castrate type. Four animals had ovaries grafted either in the peritoneal cavity or subcutaneously on the abdomen. The sex accessory glands of these animals showed no sign of male hormone effect. The presence of grafted uteri and vaginae did not effect the response. Thermocouple recordings in mice show that the temperature of the tip of the ear and the tip of the scrotum are approximately the same; i.e., about 29-30°C., and average 5-6°C below the deep body temperatures. The histology of the grafted gonads has so far given no clue to their peculiar physiology. The suggestion is made that temperature may be the controlling factor.

The effect of artificially raised metabolic rate on the electro-encephalogram of schizophrenic patients. Rubin, M. A., L. H. Cohen and H. Hoagland. To appear in this Journal.

The effect on the alpha frequency of artificially raised basal metabolic rate (by hyperthyroxinization) has been studied in 4 schizophrenic patients. In 3 of the patients the alpha frequency was increased 4.9 to 17.2% when the B.M.R. was increased 26.5 to 47.7%. The fourth patient showed an initial increase accompanying an increase in B.M.R.; after about a week, however, despite a continued high B.M.R., the alpha frequency fell to the prethyroxinization level. In general the raised metabolic rate was associated with a rise in alpha frequency. A constant high level for both these variables was maintained during thyroxin medication despite an increase in dosage and the continuation of thyroxin, and was maintained for a lengthy period after thyroxinization was discontinued. The present data, in the light of other investigations, lend further weight to the hypothesis that there exists a quantitative relationship between alpha frequency and carbohydrate metabolism of the brain.

ADRENALS

The cyclic variations of liver glycogen of adrenalectomized rats (Die Cyclischen Veranderungen im Leberglykogen von Ratten nach Nebennierenexstirpation). Agren, G., Biochem. Ztschr. **281:** 367. 1936.

The cyclic variations of the liver glycogen content observed in normal animals during a 24-hour period disappeared in 43 adrenalectomized and most likely cortin insufficient rats. There is a possible relation between cortin production and cyclic variation of the liver glycogen. Wildstrom's (unpublished) method of cortin assay, utilizing the temperature resistance of adrenalectomized rats was employed.—F. Breuer.

Neurocirculatory asthenia: Report of a case treated by adrenal denervation. Anderson, J. P., Ann. Int. Med. **9:** 1255. 1936.

The author presents a case of neurocirculatory asthenia in which bilateral adrenal denervation had been performed. Eleven years previously the patient had been subjected to a thyroidectomy (subtotal) for the same symptoms. Following the bilateral denervation polyuria developed, and the heart rate which previously averaged about 126 beats per minute was never found to be over 90. In spite of the fact that the patient lost 2 pounds per month for 1 year following the operation and developed a menstrual irregularity the author believes the operation was of considerable benefit in as much as it relieved the tachycardia, palpitation, flushing, inward nervousness, and cold extremities.—G. W. Thorn.

Effect of epinephrine on glucose excretion in fasted depancreatized dogs. Bachrach, W. H., W. B. Bradley, and A. C. Ivy, Am. J. Physiol. **117:** 203. 1936.

The purpose of the investigation was to determine whether or not it was necessary to postulate gluconeogenesis to account for the 'extra sugar' excreted after epinephrine injections in fasting, depancreatized dogs. Fourteen female dogs were used. After operation they were brought to a good state of nutrition on a carefully planned diet and insulin to keep the blood sugar below the renal threshold. After 2 days of fasting the bladder was emptied and urine collections made in 3 consecutive 12 hr. periods during the third of which 1 cc. of 1:1000 epinephrine was injected subcutaneously every 3 hr. Urinary sugar was determined by the Shaffer-Hartman method and total nitrogen by that of Kjeldahl. After taking into

account the 'extra glucose' obtainable from protein, glycerol of fat and muscle glycogen computed to have been lost during the experiment, there was no need to postulate gluconeogenesis from fatty acids to account for the 'extra sugar' excreted during epinephrine administration. In fact it could all have come from the muscle glycogen alone. Recent literature on the subject is reviewed and compared.—K. A. Brownell.

Continuous intravascular injections of adrenalin in dogs. The minimal dose inducing hyperglycemia and the route of administration (Injections intra-vasculaires continues d'adrénaline chez le chien. Recherche de la dose limite hyperglycémiante pour différentes voies d'introduction). Baudouin, A., H. Bénard, J. Lewin and J. Sallet, Compt. rend. Soc. de biol. **121:** 1157. 1936.

The minimal active dose of adrenalin producing hyperglycemia in 8 dogs was approximately 0.01 mg. per kg. per hour when the adrenalin was introduced into the body by way of peripheral veins. As much or more adrenalin was required to induce hyperglycemia in 11 dogs injected intra-arterially and in 9 dogs injected intravenously via the mesenteric veins.—E. Cutuly.

Effect of suprarenalectomy on pH and work capacity of muscle (L'effet de la surrénalectomie sur le pH et le pouvoir tampon du muscle). Benetato, G., Compt. rend. Soc. de biol. **121:** 1547. 1936.

A study on the isolated gastrocnemius muscle of 9 normal and 14 suprarenalectomized frogs showed that at pH 7.3-6.3 the work capacity of muscle from decapsulated animals was ½ or ⅗ normal. This diminished value was said to be due partly to a decrease in muscle phosphagen and partly to protein modifications.—E. Cutuly.

Effect of suprarenalectomy on the colloidal state of muscle proteins (L'effet de la surrénalectomie sur l'état colloïdal des protéines musculaires). Benetato, G., and R. Oprean, Compt. rend. Soc. de biol. **121:** 1551. 1936.

In the muscle of suprarenalectomized frogs there occurs a modification of the colloidal state of proteins through a decrease in their 'solubility.' These changes are thought to be due to metabolic disturbances and to be the immediate factors concerned in the profound modification of muscle function.—E. Cutuly.

The relation of sex hormones in a case of virilism by hypernephroma. Bruins Slot, W. J., Acta med. Scandinav. **89:** 371. 1936.

In the urine of a female patient with virilism, a determination of the male hormone by the comb growth method showed 2200 units, with a normal amount of F. S. H. and gonad stimulating hormone. On operation a hypernephroma of the left adrenal was removed, which was found not to contain abnormal amounts of male or female hormones.—J. M. L.

On carbohydrate metabolism in adrenalectomized animals. Buell, Mary V., Ian A. Anderson, and Margaret B. Strauss, Am. J. Physiol. **116:** 274. 1936.

A study was made of the distribution of lactic acid and related compounds in alimentary tract, liver, muscle and blood of adrenalectomized, 24 hr.-fasted, male rats, 3 hr. after the administration of a known quantity of neutralized d-lactic acid by stomach tube. In all, 90 animals were used, divided into 4 groups as follows: normal animals, adrenalectomized animals maintained in a state of chronic insufficiency by the use of inadequate amounts of adrenal extract, adrenalectomized animals kept in good condition by the substitution of salt solution for drinking water and adrenalectomized animals maintained in good health by the oral administration of a potent charcoal adsorbate prepared from beef adrenals. It was found that the livers of adrenalectomized rats failed to remove d-lactic acid from the blood at a normal rate and to convert it into liver glycogen. The rate of absorption of d-lactic acid from the gastro-intestinal tract of rats in adrenal insufficiency was definitely slower than in normal rats. Adrenalectomized rats, fasted 24 hr., all possessed low carbohydrate stores in blood and liver whether they showed symptoms of adrenal insufficiency or were in good condition as a result of the ingestion of salt water or active charcoal adsorbate. Adrenalectomized rats, maintained in good condition with charcoal adsorbate, made excellent use of ingested d-lactic acid. The errors in carbohydrate metabolism, resulting from adrenalectomy do not per se, necessarily cause rapid death in the rat, protected by salt from excessive loss of water and electrolytes.—K. A. Brownell.

The preparation of extracts containing the adrenal cortical hormone. Cartland, G. F., and M. H. Kuizenga, J. Biol. Chem. **116:** 57. 1936.

The cortical hormone was prepared from whole beef adrenal glands by extraction with neutral acetone and removing inert fatty substances with petroleum ether. The active principle is then taken up in ethylene dichloride to separate it from epinephrin and phospholipids. After removal of the ethylene dichloride by vacuum distillation, the residue is dissolved in dilute ethyl alcohol and extracted with petroleum ether to remove cholesterol. The final product is dissolved in 0.9% saline and sterilized by Berkefeld filtration. It assays about 2500 dog units per kilo of fresh gland and is substantially free of epinephrin.—J. M. L.

An effect of adrenalin on liver suspensions (Ueber eine an Leberaufschwemmungen beobachtete Wirkung des Adrenalins). Geiger, E., Biochem. Ztschr. **281:** 86. 1935.

It has shown that neither high nor low adrenalin concentrations (10^{-6} to 10^{-16}) and varying pH cause more rapid glycogen degradation or increased sugar formation of liver pulp. It was, however, found that by the addition of adrenalin to liver pulp containing glycogen the sedimentation rate of trichloracetic acid protein precipitate was markedly increased. Since changes in enzymatic glycogen degradation could not be detected by the common chemical methods, it is assumed that adrenalin addition reduces the protective colloid action of glycogen. —F. Breuer.

Six cases of Addison's disease. Hanssen, P., Acta med. Scandinav. **89:** 426. 1936.

The symptoms and signs of Addison's disease and the details in the case histories of 6 patients are discussed. The results of treatment with high salt diets and adrenal cortex preparations are given. Three of the patients died despite treatment. Loss of weight, decrease of blood pressure in the upright position, and morning nausea and vomiting are stressed as diagnostic features.—J. M. L.

The presence of glucose in the salivary secretion after the administration of adrenalin. Hebb, Catherine O., and G. W. Stavraky, Quart. J. Exper. Physiol. **26:** 141. 1936.

Many workers have reported that the salivary glands are practically impermeable to glucose. This work deals with the effect of adrenalin on the permeability of the submaxillary glands of the cat to glucose. Eighteen animals fasted 18-24 hours and placed under nembutal anaesthesia were used. Injections were made into the femoral vein and blood was drawn from the femoral artery. The submaxillary duct was cannulated. Fermentable reducing substances were determined in the saliva and were assumed to be glucose. Glucose was not present under ordinary conditions in the submaxillary saliva secreted in response to sympathetic or chorda tympani stimulation, unless the blood sugar was higher than 400 mg. %, but was present following adrenalin injection. Since the amount injected was not sufficient to raise the blood sugar to this level, the authors concluded that the effect was not due to hyperglycaemia, but to the action of adrenalin on the gland itself.—G. Toby.

The water and electrolyte distribution among plasma, red blood cells and muscles after adrenalectomy. Hegnauer, A. H., and E. K. Robinson, J. Biol. Chem. **116:** 769. 1936.

Water, sodium, potassium and chloride were determined in the plasma, red blood cells and muscle of 8 cats before and after adrenalectomy. There was no change in plasma osmotic pressure as determined by the freezing point, although both Na and Cl were greatly reduced. The plasma was hypotonic to normal red cells. The red cells of the operated animals showed a decrease in cations due to a loss of Na, and an increase in K and water, hence the red cells were permeable to cations. The amount of K in muscles showed an increase in terms of dry weight, hence the high K content of plasma was not due to its liberation from the muscles.—J. M. L.

Influence of cortical hormone on liver glycogen (Der Einfluss von Nebennierenrindenhormon auf den Glykogengehalt der Leben). Hochfeld, H. A., Biochem. Ztschr. **282:** 392. 1935.

The question of a conversion of fat into sugar was studied in rats. The animals were fed on normal or fat diet and the glycogen was determined following a short period of fasting. Liver glycogen was slightly higher after fat diet than after normal diet. It was further increased about 50% on the average by injections of cortin (1 injection on 3 successive days, but not during fasting period, 6 animals) if the animals were on a fat diet, but no significant effect was produced by cortin if the animals were on a normal diet (5 animals). The effect was still greater (3 animals) if pure fat was given for 3 days and if

cortin was injected every day including the fasting period. These results are considered as evidence for a conversion of stored fat into carbohydrates.—E. Bozler.

Masculinizing action of water soluble extracts of beef adrenals in the guinea pig (Action masculinisante d'extraits hydrosolubles de surrenales de boeuf sur le cobaye). Hodler, Mlle D., Compt. rend. Soc. de biol. **122:** 512. 1936.

Water soluble extracts from the cortex of beef adrenal glands when injected into normal female guinea pigs caused hypertrophy of the clitoris, disturbances in the estrous cycle, and adrenal hypertrophy; such extracts had but a slight masculinizing action in a spayed guinea pig. Injection of adrenal extract into a castrated male guinea pig prevented atrophy not only of the external genitalia but also of the seminal vesicles and prostate.—E. Cutuly.

Work capacity of the adrenalectomized rat treated with cortin. Ingle, D. J., Am. J. Physiol. **116:** 622. 1936.

Rats which were subjected to sham operations were compared to adrenalectomized rats, both with and without cortin treatment, for ability to withstand work and anesthesia. The animals were anesthetized with phenobarbital sodium, and the gastrocnemius muscle was stimulated to lift a 100 gm. weight 3 times each second. Administration of cortin to the adrenalectomized animal enhanced its work capacity in proportion to the amount of cortin administered until an optimal dosage was reached. The average performance of the rats treated with cortin remained below that of animals whose adrenal bodies were intact.—Author's Summary.

The adrenal cortical syndrome in a case with tumor from an accessory adrenal gland. Josephson, B., Acta med. Scandinav. **90:** 385. 1936.

A boy 17, showing typical symptoms characteristic of pituitary basophilism, including obesity, infantilism, hypertension, striae atrophicae, and albuminuria, was operated on for an adrenal tumor. The adrenal glands were both hypoplastic, but the tumor had originated in an accessory adrenal on the left. Removal of the tumor resulted in an acute adrenal insufficiency and very low blood pressure. Death occurred despite the administration of large amounts of glucose, salt, and cortin.—J. M. L.

Adrenalin secretion in hypophysectomized dogs (L'adrénalino-sécrétion chez les chiens hypophysectomisés). Képinov, L., Compt. rend. Soc. de biol. **122:** 351. 1936.

The adrenalin content of blood in the suprarenal veins of 4 hypophysectomized dogs was greater than that of 3 normal dogs. Injections of large amounts of insulin caused both in the hypophysectomized and normal dogs a lowering of blood sugar and an augmented adrenalin output. These results incline the author to the belief that adrenalin alone cannot induce hyperglycemia, but that mobilization of glucose is produced by a synergistic action between adrenalin and a hypophyseal hormone.—E. Cutuly.

The adrenal factor in carbohydrate metabolism. Levy Simpson, S., Proc. Roy. Soc. Med. **24:** 36. 1936.

Although injections of cortical extract did not influence the blood sugar concentration in man (normal, Addison's disease and diabetes), it replenished the low glycogen content of the liver, and muscle, in the adrenalectomised animal. The coincident diminution of lactic acid in the blood suggested that the conversion of the latter into liver glycogen was a function of the adrenal cortex. That adrenalin was secreted in insulin hypoglycaemia was indicated by the increase in erythrocytes, leucocytes, percentage polymorphonuclear cells, and platelets, as well as the disappearance of eosinophilia in a male adult, as similar changes follow adrenalin injections.—Author's Abst.

Treatment of Addison's disease with salt. Levy Simpson, S., Proc. Roy. Soc. Med. **24:** 45. 1936.

Salt therapy is usually, but not invariably, of value in Addison's disease. In some cases its emetic action prevented its use, but cortical extract injections resulted in improvement. In 2 cases of crisis, when salt was without benefit, cortical extract produced recovery. The addition of salt therapy is of value when the dose of cortical extract is inadequate. Signs and symptoms of adrenal insufficiency may occur in spite of normal serum values for sodium, chloride and potassium.—Author's Abst.

Chemical studies of the suprarenal cortex. II. The identification of a substance which possesses the qualitative action of cortin; its conversion into a diketone closely related

to androstenedione. Mason, H. L., C. S. Myers and E. C. Kendall, J. Biol. Chem. **116:** 267. 1936.

By fractionation of extracts of suprarenal gland between benzene and water, a compound 'E' was obtained which has the same qualitative physiological activity as cortin but has no progesterone activity. As it shows a maximum absorption of 2370Å it contains an αβ unsaturated carbonyl group and appears to be a trihydroxydiketone with a probable structure $C_{21}H_{28}O_5$. When oxidized with chromic acid, it yielded a ketone, $C_{19}H_{24}O_3$, which stimulated comb growth in the capon. Since this is presumptive evidence that the degradation product of the trihydroxydiketone contains a sterol ring system, cortin-like activity is associated with the sterol ring system in the trihydroxydiketone. It is proposed that cortin also must contain a sterol ring structure. A provisional formula for compound E is given as 3.20-diketo-17, 21, 8 or 14, trihydroxy-Δ4, 6 pregnanediene.—J. M. L.

A study of the blood sugar of the adrenalectomized dog. Parkins, W. M., H. W. Hays, and W. W. Swingle, Am. J. Physiol. **117:** 13. 1936.

The problem under investigation was the significance of the blood sugar level as a determining factor in the course of adrenal insufficiency. In only 4 cases of 28 adrenalectomized animals in collapse after withdrawal of cortical extract, was the blood sugar below normal limits and these were balanced by an equal number in which there was an actual rise in blood sugar. Dogs bilaterally adrenalectomized at a single operation (8 experiments) and traumatized, adrenalectomized animals (6 cases) may show sharp reductions in blood sugar when in collapse. The blood glucose changes in these types of experiment are, however, extremely variable. Adrenalin, injected into animals revealing a tendency for the blood sugar to fall when subjected to these procedures, raises the sugar to normal or considerably above but has no effect on the shock symptoms. Intravenous or intraperitoneal injections of large amounts of cortical hormone have no significant effect upon the blood sugar level of healthy adrenalectomized dogs or of animals prostrate from insufficiency. Intraperitoneal injections of isotonic glucose into healthy, adrenalectomized dogs on a maintenance dose of hormone induce shock and collapse. Animals so treated (7 experiments) die, unless injected with cortical extract or concentrated salt, with blood sugar levels far above normal. The adrenalectomized bitch in estrus (pseudopregnancy) maintains herself in normal health for 40 to 60 days without cortical hormone. During this interval the blood glucose is generally higher than normal. The adrenal cortical hormone per se is apparently not directly concerned with the metabolism of carbohydrate, at any rate, in so far as this is reflected by changes in the blood glucose levels of the adrenalectomized dog.—K. A. Brownell.

The production of sympathin in response to physiological stimuli in the unanesthetized animal. Partington, P. P., Am. J. Physiol. **117:** 55, 1936.

After denervation of the adrenal glands various stimuli producing sympathetic activity, such as emotional excitement, hypoglycemia and cold, caused contractions of the sensitized nictitating membrane in unanesthetized cats. Complete sympathectomy abolished these responses. It is concluded that these contractions are due to circulating sympathin liberated in physiological conditions during sympathetic activity.—Author's Summary.

The salt and water metabolism of adrenal insufficiency and partial starvation in rats. Rubin, M. I., and E. T. Krick, J. Clin. Investigation **15:** 685. 1936.

Young white rats were bilaterally adrenalectomized. Of these 80% died if no therapy was used; the others had demonstrable accessory adrenal tissue. Results of saline therapy were compared with paired animals on similar food intakes. The mineral balances were studied for sodium, potassium, calcium, magnesium, and chlorin, as well as for nitrogen and phosphorus. All showed negative balances on the occurrence of adrenal insufficiency, but the balances were similar in the case of partial starvation save in the case of sodium and potassium and of chlorin. Here there was a greater negative balance than the diet could account for. There was a loss of potassium, out of proportion to nitrogen from tissue loss, but associated with a diuresis, i.e. probably from tissue fluid. The authors consider the cortical hormone influences more processes than sodium, potassium, and water balance.—E. L. S.

Peculiar granules in the cells of the liver and adrenal in infections. F. L. Santee, Bull. Johns Hopkins Hosp. **59:** 427. 1936.

Patients who die of severe infections very frequently showed at autopsy conspicuous oval or elongated granules in the cytoplasm of the cells of the liver and adrenal cortex.—A. G.

Production of alarm reaction with follicle hormone (Über die Auslösung der Alarmreaktion mit Follikelhormon). Selye, H., C. Harlow and J. B. Collip, Endokrinologie **18:** 81. 1936.

One of the principal symptoms of the alarm reaction is the acute involution of the thymus. This follows various, non-specific, harmful influences only in the presence of the adrenal. It also has been shown that high dosage of follicle hormone can cause a thymus involution which is more chronic in nature, in both male and female rats. Likewise other symptoms characteristic of the alarm reaction (ulceration of gastric mucosa, adrenal hypertrophy, lymph node involution) can be produced with follicle hormone. Large doses of follicle hormone have marked toxic actions, especially in hypophysectomized and adrenalectomized rats, in which an elevation of the animal's resistance through thyroid and adrenal hyperplasia cannot occur.—W. J. A.

The technique of suprarenalectomy in the guinea-pig and the survival period and cause of death of guinea-pigs after bilateral suprarenalectomy. Simmons, H. T., and R. Whitehead, J. Physiol. **88:** 239. 1936.

A method for removing adrenals from guinea-pigs, by resection of the lowest rib, is given. Control operations indicated that death within 3 days of operation was probably due to trauma and after 3 days to adrenalectomy. A total of 189 animals was used.—R. L. Z.

Experiments on intact and adrenalectomized dogs subjected to sodium and chloride depletion by intraperitoneal injections of glucose. Swingle, W. W., W. M. Parkins and A. R. Taylor, Am. J. Physiol. **116:** 430. 1936.

Removal of NaCl by paracentesis following intraperitoneal injection of glucose renders normal dogs more sensitive to hemorrhage. However spontaneous recovery occurs after hemorrhage of 10 cc. per kg. Adrenalectomized dogs on maintenance cortin die from the intraperitoneal injection of only one-half the amount of glucose withstood by a normal animal unless concentrated (30%) sodium chloride is injected intravenously or large doses of cortin are used. The adrenalectomized dog prostrate from insufficiency is the physiological counterpart of the adrenalectomized dog in collapse following intraperitoneal glucose injection. Both types are unable to restore plasma volume. Adrenalectomized dogs primed with large amounts of cortin react much like normal animals. Adrenalectomized dogs in collapse from glucose injections can be revived by a single intravenous injection of 30% sodium chloride or by injection of cortin. Recovery is attended by restoration of plasma.—F. A. H.

Relation of serum sodium and chloride levels to alterations of body water in the intact and adrenalectomized dog, and the influence of adrenal cortical hormone upon fluid distribution. Swingle, W. W., W. M. Parkins, A. R. Taylor and H. W. Hays, Am. J. Physiol. **116:** 438. 1936.

Five adrenalectomized dogs were studied through a cycle of insufficiency and recovery while maintained at low sodium and chloride levels. The clinical condition is independent of serum sodium and chloride levels when adequate amounts of cortin are administered. The shift of fluid into the blood stream occurs despite the low serum sodium and chloride. The serum sodium and chloride concentrations do not change. Water balance studies on 4 animals showed that there was a smaller loss of fluid by way of the urine during insufficiency than during recovery with extract in such animals. Urinary excretion of sodium and chloride in salt-depleted dogs during insufficiency is very small. Intact dogs, depleted of sodium and chloride by intraperitoneal injections of isotonic glucose, can be maintained healthy and vigorous for 3 weeks with extremely low levels of serum sodium and chloride. Sufficient cortical hormone must be elaborated by the animal's own adrenals to restore the altered fluid distribution to approximately normal condition without change in the level of the serum sodium and chloride. Injections of hormone, supplementary to that secreted by the animal's own glands, rapidly reduces the hemoconcentration and blood urea nitrogen to normal.—Author's Summary.

Hypoglycemia in Addison's disease. Welty, J. W., and H. F. Robertson, Am. J. M. Sc. **192:** 760. 1936.

Two cases are reported in which coma that could be relieved by dextrose was a striking manifestation. In one of the cases the blood chloride level was approximately normal. It is suggested that hypoglycemia in such cases is probably due to involvement of the medulla.—R. G. H.

Chemical studies on the adrenal cortex. III. Isolation of two new physiologically inactive compounds. Wintersteiner, O., and J. J. Pfiffner, J. Biol. Chem. **116:** 291. 1936.

An αβ unsaturated diketone was obtained by fractional crystallization of the insoluble residue left from the chloroform extracts of adrenal gland. It was separated from other crystalline fractions by the use of betaine-hydrazide hydrochloride. It was given the formula $C_{21}H_{28}O_2$. It showed an intense green fluorescence when dissolved in concentrated sulfuric acid, and had a maximum absorption of 2400Å which is characteristic of αβ unsaturated ketones. No cortin activity was found when assayed on 3 adrenalectomized dogs, nor progesterone activity when assayed by the rabbit method. The second compound was a monoketone of the probable composition $C_{21}H_{34}O_2$.—J. M. L.

Adrenal glands in dogs with high intestinal obstruction. Wohl, M. G., J. C. Burns, and J. H. Clark, Proc. Soc. Exper. Biol. & Med. **33:** 543. 1936.

In dogs in which an experimental high intestinal obstruction had been produced, it appeared that there was some loss of lipoid in the cells of the adrenal glands.—M. H. F.

ENDOCRINE GENERAL

Further observations on the chemical nature of a hematopoietic substance occurring in the liver. Dakin, H. D., C. C. Ungley and R. West, J. Biol. Chem. **115:** 771. 1936.

The hematopoietic substance of liver was fractionated by precipitation with ammonium sulfate, basic lead acetate, and Reinecke acid and subsequent regeneration. The amino-hexose previously described was not an essential component, but arginine, leucine glycine, proline, hydroxyproline, aspartic acid and an acid resembling hydroxglutamic acid were produced by hydrolysis. The active material is, or is associated with, a peptide possessing some of the properties of an albumose, and ultrafiltration experiments indicate a molecular weight between 2000 and 5000. It was rapidly denatured by NaOH but not by NH_4OH, and was not hydrolized by depepsinized gastric juice. The active material was not obtained from kidney or brain. It is essentially different from the materials described by Subbarow or by Colin. —J. M. L.

Effects of excessive doses of vitamin D on the histological structure of the glands of internal secretion (Les effets des fortes doses de vitamine D sur la structure histologique des glandes à sécrétion interne). Nitzesco, I., and S. Bratiano, Compt. rend. Soc. de biol. **121:** 1533. 1936.

Five dogs were used. The thyroid showed signs of hyperactivity. In the testis there was profound atrophy of the tubules and degeneration of the interstitial cells. Intense congestion was found in the hypophysis; the eosinophiles were markedly increased in number in the gland. The thymus was congested, contained few thymocytes and many small corpuscles of Hassall. Varying degrees of congestion were noted in the lymphatic ganglion, spleen, pancreas, liver and suprarenal.—E. Cutuly.

Anti-endocrine gland precipitins of human blood serum (Considérations préliminaires sur les précipitines antiglandes endocrines du sérum humain dans leur ensemble). Picado, C., Compt. rend. Soc. de biol. **121:** 1146. 1936.

Glands from guinea pigs are triturated in saline solution, centrifuged and filtered. The filtrate is a fine emulsion of glandular material, free of precipitates. Human blood serum from normal individuals and from subjects with endocrine disturbances when added to the various glandular emulsions produces varying degrees of precipitation as determined by an agglutinoscope. Diagnosis of existing or past endocrine disturbances is said to be possible on the basis of comparative differential precipitations. Details of the technique, results obtained, and a discussion of results are presented.—E. Cutuly.

Dihydroxyphenyl ethanolamine (arterenol) as a possible sympathetic hormone. Stehle, R. L., and H. C. Ellsworth, J. Pharmacol. & Exper. Therap. **59:** 114. 1937.

Arterenol, injected into decapitated cats which had received ergotamine, produced an increase in blood pressure. From these results the authors believe that arterenol may be the pressor substance liberated by the stimulation of the hepatic sympathetic nerves.—E. L.

Effect of restriction of inorganic salts in the diet on organ growth. Swanson, P. P., and A. H. Smith, Am. J. Physiol. **116:** 516. 1936.

A deficiency of inorganic materials in the diet manifests itself in two ways on the development of the organs. First, a short period of subnormal growth may occur that is followed by a distinct regression in weight. As a result, the organs may weigh less at the end of the experiment than do the same members in normal rats of similar body weight. The heart and spleen are examples of organs thus affected. Second, there are those organs whose rate of growth is at first abnormally accelerated by the absence of dietary salts but which later undergo a shrinkage. Of these, the kidneys, the testes, and the adrenals are examples. The testes show a loss in weight by the time the experiment is concluded. In spite of the shrinkage that occurs, the kidneys and adrenals are larger than the same organs in normal animals of equal size. The possible relation of adrenal hormone to the regulation of salt metabolism has been pointed out on the basis of the correlation of changes in size of the adrenal and shifts in the distribution of water in blood and muscle.—Author's Summary.

Correlations of the endocrine system. Thomson, D. L., Ann. Int. Med. **9:** 864. 1936.

This article is a concise review of the known interrelations of the endocrine system. It introduces no new material, but brings the discussion up to the recent hypothesis of the 'anti-hormones.'—G. W. Thorn.

On the presence of anti-anemic activity in the ileal part of the small intestine. Uotila, U., Acta Med. Scandinav. **89:** 50. 1936.

Sections of the ileum 1 m. from the ileocecal junction were wished free from intestinal contents, minced and dried at 38 to 40° C. The dry residue was extracted with benzine and powdered. Two patients with pernicious anemia showed marked improvement when fed 40 gm. daily of this material. The activity of the ileal preparations was about 50 to 60% of that of dried stomach powder.—J. M. L.

Influence of hormones on the alcohol metabolism (Hormonale Einflusse auf den Alkoholumsatz). Widmark, E. M. P., Biochem. Ztschr. **282:** 79. 1936.

The author measured the rate of decrease of alcohol in blood in dogs and determined the influence of thyroxin, pituitrin, adrenalin and insulin on this process. Only insulin was effective and increased the rate of disappearance of alcohol during the first 200 minutes after administration as much as 200%. This effect greatly varied in different experiments, even on the same animal; it was greatest if the rate of disappearance of alcohol was low initially. These variations might be due to the different quantities of insulin already present in the body.—E. Bozler.

GONADS

The effect of progesteron on male castrates (Über die Wirkung von Progesteron auf männliche Kastraten). Albrieux, A., W. Buno, P. Engle and N. Morato-Manaro, Klin. Wchnschr. **15:** 206. 1936.

The daily injection of castrate male rats for 14 days with as much as 7 Clauber Rb.U. of purified progesteron failed to influence the castration changes in the male accessories, or in the pituitaries. The preparation also failed to induce any change in the comb of the capon.—M. H. F.

A simplified method for the preparation of crystalline progesterone from pig ovaries. Allen, W. M., and C. Goetsch, J. Biol. Chem. **116:** 653. 1936.

Pure crystalline progesterone was prepared from pig ovaries by extracting with methyl alcohol and reclaiming the active principle in a continuous extractor with petroleum ether. The crude material from the petroleum ether was recrystallized from dilute pyridine yielding a pure product containing 25% of the hormone present in the tissues.—J. M. L.

Further studies of sex skin reactions in Macaca mulatta. Bachman, C., J. B. Collip and H. Selye, Proc. Soc. Exper. Biol. & Med. **33:** 549. 1936.

The daily injection of 500 gamma of crystalline-folliculine (oestrone; Girard) into 3 castrate females and 2 adolescent, acyclic females produced results similar to those obtained with oestrio. The genital reactions appeared on the 2nd day and reached a maximum by the 16th day, after which the color varied for some days and then began to fade despite continued injections. From the 20th day onward, an extra genital oedema appeared and spread over the hips, thighs, legs, back and brow. Eight days following the cessation of the injections (injections were continued for 48 to 76 days), vaginal bleeding occurred and lasted 3 days. In one of the

animals a piece of reactive skin from the thigh which had been transplanted to the abdomen reacted normally to the injections, although a piece of abdominal skin transplanted to the genital region failed to participate in the reaction.—M. H. F.

Action of folliculin on the liver (Action de la folliculine zur de foie). Baltaceano, G., and C. Vasiliu, Compt. rend. Soc. de biol. **121:** 1541. 1936.

Folliculin administered to dogs caused an increase in the volume of the liver and a diminution in the amount of bile secreted. The liver while in a distended condition alternately contracted and expanded energetically. These findings suggest a cause for hepatic congestion, diffuse pains, nausea, etc., encountered clinically.—E. Cutuly.

Excretion of sex hormones in urine (Untersuchungen über die Ausscheidung von Keimdrüsenhormonen im Urin). Bingel, A., Klin. Wchnschr. **14:** 1827. 1935.

Assay of extracts of urine for male hormone by means of direct application of the extract in olive oil to the capon's comb proved to be 50 times as sensitive as the usual method of injection of such material. Examination of urine of a normal male by this method revealed an excretion of 500 to 800 U. of male hormone over a 3-day period (100 to 250 per liter). Using this method of examination for the male hormone and the conventional method for detecting female hormone it was found that old people of both sexes excrete small amounts of both hormones in the urine (male hormone, 20 to 80 U. and female hormone, 0 to 10 M.U. per liter). A 67-year old male, recently castrated, excreted 30 M.U. of female, and 3 to 6 U. of male hormone daily. Although it is estimated that such amounts could be accounted for by the hormone content of the ingested diet (allowing for excretion of 5% of the intake), attention is called to Zondek's inability to influence this rate of excretion by changes in diet. It is concluded that the demonstration of either sex hormone in the urine is no proof of the presence of a functional gonad.—M. H. F.

Quantitative study on the antagonism between the two crystalline hormones of the ovary (Etude quantitative de l'antagonisme humoral ovarien effectuée avec les deux hormones cristallisées). Courrier, R., Compt. rend. Soc. de biol. **122:** 661. 1936.

Female rabbits were spayed 24 hours after mating and then were injected for 6 days with a total of 1.5 mg. of synthetic progesterone alone or simultaneously with varying doses of folliculin (estradiol). It was found that the progestational proliferation of the uterine mucosa produced by 1.5 mg. of progesterone alone was prevented by as little as 0.02 mg. of folliculin.—E. Cutuly.

Feto-placental dissociation in the cat caused by castration. Endocrine function of the placenta (Dissociation foeto-placentaire réalisée par la castration chez la chatte. Action endocrinienne du placenta). Courrier, R., and G. Gros, Compt. rend. Soc. de biol. **121:** 1517. 1936.

Castration of cats a few days after implantation of the fertilized egg caused death of the embryos which were resorbed. The placentae, however, continued to develop for at least 20 days. The endometrium of these animals showed the usual modifications of pregnancy. This fact strongly suggests that the placenta can substitute for the corpus luteum. A microscopic study of the maternal placentae unaffected by castration revealed in the stroma edematous areas resembling hydatidiform moles. The theory is advanced that hydrops formation results from an insufficiency of corpus luteum hormone.—E. Cutuly.

Ability of folliculin to transform the right rudimentary gonad (potential testicle) of hens into ovary (Sur la capacité de la folliculine à transformer, chez la poule, le rudiment gonadique droit [testicule potentiel] en ovaire). Dantchakoff, Vera, Compt. rend. Soc. de biol. **122:** 168. 1936.

Injections of folliculin into genetically female chick embryos caused proliferation of the coelomic epithelium in the region of the rudimentary right ovary. In adult birds this induced structure resembled Bidder's organ of amphibians. It contained ova which were structurally normal. The functional left ovary, however, better vascularized than the right, produced such an abundance of eggs that possible functioning of the right ovary was checked. —E. Cutuly.

Influence of follicular hormone on transplanted hypophysis (Influence de l'hormone folliculaire sur l'hypophyse transplantée). Desclin, L., and C. Grégoire, Compt. rend. Soc. de biol. **121:** 1366. 1936.

Homoplastic grafts of the hypophysis of adult male and female rats were made in the kidneys of normal and castrated hosts, the donors likewise being normal or castrated. All of the hosts (totalling 45) were sacrificed 15 days after the graft was made. Some of the hosts were injected with 100 R.U. of dimenformon daily for 4-5 days. Normal hypophyses transplanted to normal hosts showed an increase in the number of eosinophiles. No castration cells were found in the pituitaries of normal or castrated rats, grafted into normal or castrated animals. The injection of dimenformon into hosts caused in grafted hypophyses, as well as in the animals' own pituitaries, the appearance of numerous atypical basophilic cells. It was concluded that the female sexual hormone can exert a direct effect on the hypophysis.—E. Cutuly.

Observations on recent investigations of the male sex hormones. Ehrenstein, M., South. M. J. **30:** 229. 1937.

This is a brief but thorough presentation of the sex hormone problem from the chemical-physiological point of view.—J. C. D.

Corpus luteum cyst simulating pregnancy clinically and by hormone test (Ueber eine klinisch und hormonal durch corpusluteumcyste vorgetäuschte Extrauteringravidität). Ehrhardt, K., and H. Kramann, Zentralbl. f. Gynäk. **60:** 2642. 1936.

A woman with previously regular menses missed a period, suspected pregnancy, gave a positive Aschheim-Zondek test, and also had a pelvic tumor in the adnexa besides a slightly large uterus. At laparotomy no pregnancy was found, but the cyst was found to contain some luteinized cells. The A.Z. test was gradually reduced soon after.—E. L. S.

Folliculin in the castrate organism (Über das Follikulin im kastrierten Organismus). Eng, H., Klin. Wchnschr. **15:** 349. 1936.

Small amounts of estrin were found in castrates of both sexes (7 cases), and among them was 1 castrate male on a folliculin-free diet who continued to excrete estrin in undiminished amounts. Estrin was also found in the urine of a gelding, and in the urine of castrate male rats.—M. H. F.

Gonadotropic hormone in blood and urine of early pregnancy: Normal occurrence of transient extremely high levels. Evans, H. M., Clara L. Kohls and D. H. Wonder, J.A.M.A. **108:** 287. 1937.

The authors report what they believe to be the invariable occurrence of the early-peak phenomenon as regards the gonadotropic content of blood and urine in all normal human pregnancies—a transient and remarkable rise in gonadotropic hormone followed by a fall to values of a few thousand units per liter. This transient hormone concentration at its extreme height equals or exceeds that reported in the blood, urine and cerebrospinal fluid in hydatidiform mole and chorionepithelioma, one of the authors' cases showing a daily hormone output of 1,040,000 R.U. The lowest peak encountered in the 6 cases in which titrations were done when the hormone was at its maximal value indicated a daily output of 75,000 units—well over twice that recognized by Zondek as 'normal.' From the time of its first appearance, the increase in the concentration of gonadotropic hormone in the urine is spectacularly rapid, until a peak is reached some time between the twentieth and the fiftieth day, usually about the thirtieth day. The amount of hormone excreted at the peak seems to differentiate cases into two groups: one in which total unitage per day is perhaps between 75,000 and 150,000 R.U., and another group in which it is between 750,000 and 1,000,000. Equally remarkable is the abrupt decrease in the concentration of gonadotropic hormone after the maximum has been reached. In all but one case the hormone concentration is below 10,000 R.U. per liter by the sixty-fifth day and continues at or below this level until the termination of pregnancy. By the fifth day following parturition there is no trace of the hormone in the urine. The urinary 'peak phenomenon' is paralleled by the hormone levels of the blood. The great difference in hormone concentration between the two groups cannot be accounted for on the basis of the sex of the fetus, for patient 1 with a maximal daily excretion of 75,000 R.U., patient 2 with over 1,000,000 and patient 3 with 100,000 all gave birth to females. Also the factor of previous pregnancies is not involved.—Abst., A. M. A. Service.

The endometrial vascular bed in relation to rhythmic uterine motility, with a consideration of the functions of the intermittent contractions of oestrus. Fagin, J., and S. R. M. Reynolds, Am. J. Physiol. **117**: 86. 1936.

The density of the endometrial vascular population was studied histologically in material removed by a quick-freezing method from ovariectomized rabbits, both untreated and estrin-injected. An orderly change in vascularity occurred as a result of estrin injection, as demonstrated by the fact that in untreated animals motility was entirely absent and the number of open small vessels was high, whereas upon estrin administration the vessels dilated and their permeability was increased. The endometrium became edematous and the density of the vascular population became less. The edematous condition was attributed to the decided vasodilatation associated with an increased capillary permeability. In consequence of these changes a reduction occurred in density of the vascular population. Since these changes preceded the initiation of rhythmic uterine motility they are considered to be independent of the latter. Intermittent contractility of the myometrium, however, exerted some effect upon the vascular population, i.e., chiefly by increasing volume of blood flow through the dilated endometrial vessels, thereby preventing undue congestion. It is believed that rhythmic uterine motility during estrus serves 2 functions: the induction of an increased volume of blood flow through the vessels, and removal of some of the edema fluid. The rhythmic uterine motility of estrus, therefore, serves to limit and to repair the vascular and fluid matrix changes induced by the direct action of estrin.—Hans O. Haterius.

Augmentation of the gonad stimulating action of pituitary extracts by inorganic substances, particularly copper salts. Fevold, H. L., F. L. Hisaw and R. Greep, Am. J. Physiol. **117**: 68. 1936.

An increase in gonadotropic activity of FSH and of FSH + LH, was produced by the addition of yeast extracts, yeast ash, zinc and copper salts, as tested by the ovarian response in immature rats. Augmentation of FSH and of FSH + LH by these substances with one exception, also was induced when tested in hypophysectomized rats. Copper salts however, while augmenting the action of FSH + LH in hypophysectomized rats, were ineffective in augmenting FSH alone. This difference in activity led the authors to conclude that, while zinc may act through decreasing the rate of absorption of the active material, as had been suggested, the action of copper cannot be explained on this basis. Rather, it is suggested that the activity of copper salts lies in their catalytic action in the synergistic interaction of FSH + LH in ovarian development. It was found further that yeast extracts and copper salts induced ovulation in rabbits, although the response was somewhat delayed; zinc salts were ineffective.—Hans O. Haterius.

Oestrogenic substances in the blood and urine after castration and the menopause. Frank, R. T., M. A. Goldberger and U. J. Salmon, Proc. Soc. Exper. Biol. & Med. **33**: 615. 1936.

A study of the urine of 12 female surgical castrates, 3 x-ray castrates and 5 menopausal women, revealed an excretion of 15-200 M.U. per liter. There was no correlation of the estrogenic content of the urine with the content of gonadotropic hormone, subjective symptoms or vaginal smears.—M. H. F.

The physiology of pregnancy in the rat: further data on the passage of hormones via the placenta and the mother's milk. Hain, Annie M., Quart. J. Exper. Physiol. **26**: 29. 1936.

The injection of 0.5 mg. of estrone was capable of inducing genital abnormalities in the fetuses when injected late in pregnancy. Large amounts of estrone did not result in proportionately greater responses, although abnormalities could be made to appear earlier in fetal life. Extracts of adrenal cortex and of anterior pituitary failed to induce modifications. Compounds related chemically to estrone (benzpyrene, estrone benzoate) and the di-hydroxy derivative of androsterone induced responses similar to those obtained by estrone administration. Modifications resulted in some instances by administration of estrone even after birth. A few positive responses were evoked in suckling litters following upon administration to the mother. Vaginal smears of 'modified' females upon reaching maturity revealed no cyclic abnormalities and they appeared to be normal save for a slightly diminished fertility. Fertility was not seriously impaired in males of treated litters. It is believed that the concentration reached by the hormones rather than its duration of activity is of importance in determining the effect on fetuses.—Hans O. Haterius.

Epithelial metaplasia of cervical and uterine mucosa after administration of follicular hormone to castrate and intact rats (Zur Frage der Epithelmetaplasie der Cervix- und Korpusschleimhaut nach Zufuhr von Follikelhormon bei nichtkastrierten und kastrierten Ratten). Harold, L., and G. Effkemann, Zentralbl. f. Gynäk. **61:** 27. 1937.

Cancer-resistant rats were treated with 250 I.U. of estrin, (Perlatan) daily for 6 to 12 weeks. The group contained 15 castrates, 15 intact, and 10 untreated controls. The cervical epithelium showed areas where unicellular layers were changing to squamous epithelium of several layers. After 3 months some of the columnar epithelium was changing to a cornified multicellular layer, with inflammatory reaction around these areas. The changes occurred earlier and in more distinct fashion in the castrates. Some similar changes occurred in the endometrium. —E. L. S.

Estrogenic and gonadotropic hormones in epitheliomas of the cervix uteri (Recherches sur les hormones génitales [oestrogènes et gonadotropes] dans les épithéliomas du col de l'utérus). Imbert, R., M. Mosinger and H. Bontoux, Compt. rend. Soc. de biol. **122:** 692. 1936.

The folliculin content of blood and urine and the prolan A and B content of the urine of human subjects with cancer of the cervix uteri were determined by various tests. In 5 of 6 subjects the folliculin content of both blood and urine was greater than normal. X-ray or radium therapy caused a marked increase in excreted folliculin, with little or no change in the blood level of the hormone, indicating a threshold of excretion. An increase in prolan A excretion was found in 6 of 7 cases and an increase in prolan B in 2 of 7 cases. In general the amount of prolan A excreted was found to vary inversely as the amount of folliculin excreted; no definite correlation was found to exist between the amount of folliculin and prolan B.—E. Cutuly.

Sex hormone therapy in gynecology. Johnstone, R. W., Edinburgh M. J. **48:** 680. 1936.

The author reports on his experience in practice with estrogenic and gonadotropic hormones and with progesterone. In secondary amenorrhoea, in gonorrhoeal vulvo-vaginitis in little girls, and possibly post-menopausal kraurosis vulvae, these preparations have proved useful. In other menstrual disorders, their effect has been disappointing.—J. C. D.

Concerning the effect of insulin on the uterine muscle (Über die Wirkung des Insulins auf die Uterusmuskulatur). Klaften, E., Ztschr. f. Geburtsh. u. Gynäk. **112:** 318. 1936.

In a 10-year study, favorable influences of insulin on gynecologic conditions were noted. An explanation of the manner of action of insulin on the uterus and ovaries was sought in guinea pigs and rabbits. The effects of different preparations of insulin on the uteri and ovaries of guinea pigs and rabbits were studied. In guinea pigs, immediate and late effects were noted, the immediate effect was assumed to be direct on the uterus or vegetative nervous system and was manifested by increased tonus, increased intensity of individual contractions, shortening of resting period and increased sensitivity to pituisan. Preservatives depressed. Delayed effect was manifested by hyperemia of ovaries, increased vascularity and enlargement of the uterus with loss of total body weight. Similar extracts of thymus elicited no effect. The insulin effect on the guinea pig was entirely lacking in the rabbit. In the human no reliable test of uterine muscle reaction to physiologic conditions was found. A negative response to pituisan was not changed by insulin. In a positive pituisan response no uniform insulin reaction was noted. Sometimes there was increased tonus and sometimes increased height and frequency of spontaneous contractions. The favorable influence on menorrhagia and metrorrhagia is explained as stimulation of ovarian function and general toning of endocrine apparatus as well as favorable influence on nutrition and regenerative processes. The favorable effect on dysmenorrhea is explained as the regulating influence of uterine contractions and vegetative nervous system.—M. R. White.

Relation of cholane nucleus to the female bitterling test for male hormone. Kleiner, I. S., A. I. Weisman and D. I. Mishkind, Proc. Soc. Exper. Biol. & Med. **34:** 367. 1936.

In view of the fact that male hormone is responsible for the effect of urine on the ovipositor of the bitterling, it was deemed necessary to determine if other compounds containing the cholane nucleus would also give the reaction. Theelin and theelol sometimes caused slight increases in the length of this organ although they did not give real positive reactions. While cholesterol and ergosterol showed little or no effect, soduim taurocholate produced a fairly strong reaction when 50 mg. were added to 4 liters of water (contrasted with a similar effect of 1.7 mg. of a crude fraction of male hormone in 4 liters of water). From such results it is concluded that the cholane nucleus per se is not responsible for the reaction.—M. H. F.

A possible role of the eosinophil leucocytes in the endocrine complex of the rat. Kraatz, C. P., Am. J. Physiol. **117:** 250. 1936.

Goat anti-rat thymus serum upon intraperitoneal injection resulted in pseudopregnancy in 10 of 14 cases, confirmed in 3 instances by experimental production of placentomata. A possible gonadotropic hormone content of the immune serum was checked upon immature females. Treatment in late estrus or in postestrus lengthened the following diestrus interval; single injections during diestrus induced full estrus of 3 or more days' duration in 8 of 14 cases. Histologically, a single injection during proestrus produced a heavily leuteinized ovary and, in some cases, a single injection during diestrus produced cystic ovaries. Serum treatment invariably produced eosinophilia, a figure ranging from 6.5 to 11.5% of total W.C., whereas controls revealed an average of less than 3%. Excepting the constant estrous condition, the differences in eosinophil number paralleled the probable variation in LH liberated from the anterior pituitary. The production either of cystic ovaries or of pseudopregnancy by a common treatment, accompanied by high eosinophil counts in constant estrus are interpreted to favor the concept of a high, relatively static balance of hormonal influence in the circumstance of continuous estrus, istead of an absence of LH. Pregnant serum centrifuged and injected induced a response proportional to the number of eosinophil leucocytes present. Moreover, the concentration of leucocytes in the circulation of pregnant rats of various ages appeared to vary as the probable concentration of LH in the pituitaries.—Hans O. Haterius.

Reaction for the determination of normal and ectopic pregnancy by means of Infusoria. (Reaktion zur Bestimmung normaler und ektopischer Schwangerschaft mittels Infusionstierchen). Kustallow, N. J., Zentralbl. f. Gynäk. **61:** 269. 1937.

By mixing on a microscope slide a drop of catheterized urine and a drop of a hay infusion containing various protozoa, determination of pregnancy is made by looking for motility of the protozoa during the next 5 minutes. Normal urines do not stop the movements, while pregnant women excrete some material which stops the motions in less than 2 minutes. Under certain circumstances, such as eclampsia, infections, and ectopic pregnancy, there may be delays but not beyond 5 minutes. No percentage as to accuracy is given. Attempts to remove protein, lipoids, carbohydrates, etc. from the urine cause a diminished response.—E. L. S.

Menformon in therapy of Cushing's disease. Laqueur, E., and T. Deelen, Nederl. tijdschr. v. geneesk. **80:** 743. 1936.

During two periods of 14 days, separated by 3½ weeks' periods without treatment, comparatively large doses of estradiol benzoate were given in dosage of 50,000 or 100,000 units a day. The patient had been amenorrheic for 2 years. After the 3rd day of the treatment, there was noted: Decrease of the total number of erythrocytes, of the haemoglobin and of the fasting blood sugar. After 6 weeks, menstrual bleeding was resumed and continued for 5 additional periods at regular intervals of 4 weeks. The expression of the face, as well as the striae, were influenced beneficially. No changes were noted in the blood pressure.—L. Meyler.

Cytology of the monkey endometrium under influence of follicular and corpus luteum hormones. Lendrum, F. C., and F. L. Hisaw, Proc. Soc. Exper. Biol. & Med. **34:** 394. 1936.

A description of the distribution of glycogen, mucin and fat, in the histological sections of monkey endometrium under the influence of ovarian hormones. For other details of these sections, the reader is referred to previous articles.—M. H. F.

The isolation of the principal estrogenic substance of liquor folliculi. MacCorquodale, D. W., S. A. Thayer and E. A. Doisy, J. Biol. Chem. **115:** 435. 1936.

From 400 liters of liquor folliculi of sow ovaries, dihydrotheelin was obtained as the m-bromobenzoate and also as the di-a-naphthoate. Both derivatives were pure crystalline compounds identical with similar products derived from dihydrotheelin prepared from theelin. The composition of the naphthoate was C 82.35%.H 62.24% mol. wt. 572 which agrees well with the theoretical values for $C_{40}H_{36}O_4$. The pure hormone isolated from this compound gave analytical results agreeing with those expected from dihydrotheelin of $C_{18}H_{24}O_2$. Bio-assay of the hormone by the procedures of Marrian and Parkes, Butenandt, Allen and Doisy, and Curtis and Doisy show it to have the same estrogenic activity as dihydrotheelin.—J. M. L.

The etiology of amenorrhoea with special reference to treatment. MacGregor, T. N., Edinburgh M. J. **43:** (Transactions Edinburgh Obstetrical Society) 153. 1937.

Seventy-one cases of amenorrhoea have been studied. The underlying factors have been so divergent as to indicate that no one form of therapy meets each case. After excluding sys-

temic disturbances, the therapy to be exhibited depends largely on repeated urine analysis correlated to the uterine findings. Treatment along endocrine lines will be more successful when more potent anterior pituitary preparations are available.—Author's Summary.

The occurrence of ovulation and pseudo-pregnancy in the rabbit as a result of central nervous stimulation. Marshall, F. H. A., and E. B. Verney, J. Physiol. **86:** 327. 1936.

Electrical stimulation of the central nervous system (either brain or cord) induced ovulation in rabbits. Two stimuli (alt. current, 30 v., 50 cycles, 3 secs.), were followed by 2 more an hour later. Ovulation occurred in 7 rabbits, blood follicles in 3 of a total of 16 attempts. Pseudo-pregnancy occurred in 4 animals. Negative results were obtained from animals found at autopsy to be pregnant or not in heat. Three animals with spinal cords transected in the region of the 1st lumbar vertebrae gave negative results with stimuli either above or below the cut. Attempts to induce ovulation by drugs (pilocarpine, eserine, acetylcholine, adrenaline) known to act on the autonomic nervous system also gave negative results.—R. L. Z.

Permanent ligation of the ovary and the problem of the postnatal formation of germinal cells in the white mouse (La ligature permanente de l'ovaire de la souris blanche et le problème de la formation postnatale de cellules sexuelles chez cet animal). Martinovitch, P. N., Compt. rend. Soc. de biol. **122:** 335. 1936.

The ovaries of 34 mice aged 20-40 days were ligated at the hilus. At intervals between the first day and third month after the ligation ovaries were removed and studied microscopically. Extensive degeneration of ova occurred on the day following ligation; a number of ova in primary follicles situated near the periphery of the glands, however, remained normal. Survival of these ova was attributed to a rapid reëstablishment of blood circulation in the ovaries. Six days after ligation the ovaries showed distinct signs of rehabilitation and after 10 days they were almost normal in appearance. Similar results have been obtained on rat ovaries from which the capsule was removed before ligation.—E. Cutuly.

Studies on optimal dosage of estrogens: Experimental and clinical evaluation. Mazer, C., and S. L. Israel, J.A.M.A. **108:** 163. 1937.

In a previous study on constitutional effects of relatively large doses of estrogens, it was found that the administration of from 100,000 to 260,000 R.U. of the principle, given in divided doses over a period of from 2 to 3 months, produces no appreciable changes in body weight, basal metabolism, blood pressure, blood count, coagulation and bleeding times, blood chemistry and urine. The present study was undertaken in an attempt to determine the indications for the clinical use of estrogens, their respective optimal dosage and the most effective modes of administration. With proper dosage and sufficient length of treatment the genital and pituitary responses to estrogens can be duplicated under certain conditions in the human being. In order to determine the proper interval for the hypodermic use of estrogen, a single dose of from 1,000 to 10,000 R.U. was given to 12 surgically castrated women and the entire output of urine was extracted daily for a period of 5 days. Discounting slight individual variations, the hypodermic administration of a single dose of 1,000 R.U. of theelin or estradiol benzoate in oil maintains a normal level of estrogen in the blood, as reflected by the amount excreted in the urine, for a period of 4 days. Larger doses of from 5,000 to 10,000 R.U. produce a temporary hyperestrinemia, which invariably reaches the normal premenstrual level on the fourth or fifth day. Even 500 R.U. administered hypodermically daily produces an abnormally high concentration of the principle in the blood, as reflected by the amount excreted by the kidneys daily. Twenty-one surgically castrated women were given estrogens orally in doses of from 200 to 6,000 R.U. daily in the form of an oily solution on buttered bread, and it was observed that estrogens are readily absorbed from the human gastro-intestinal tract. The degree of absorption varies considerably with the product and the amount administered. Therapeutically, estrogen may be administered in various conditions with the following objectives in mind: 1. To overcome uterine hypoplasia resulting from a natural deficiency of estrogen, as seen in most instances of amenorrhea, hypomenorrhea and dysmenorrhea and occasionally in the dysfunctional sterility of regularly menstruating women. 2. To inhibit one or more of the several functions of the anterior pituitary lobe by inducing a constant hyperestrinemia in the treatment of such conditions as the severe menopausal syndrome, the lobular form of abnormal breast hyperplasia, premenstrual migraine, pituitary hyperthyroidism and selected cases of diabetes mellitus. 3. To produce a purely local growth effect in the vaginal mucosa of children suffering from vulvovaginitis and of postmenopausal women suffering from senile vaginitis. 4. To evoke a pituitary-ovarian response in cases of severe amenorrhea by employing intermittently massive doses, such as 300,000 R.U. over a period of one week.—Abst., A. M. A. Service.

Germinal response (in male mice) to environmental conditions. Ogle, Cordelia L., Am. J. Physiol. **117:** 285. 1936.

Constant humid heat (88-92°F.) caused degeneration of the germinal epithelium in male mice, together with giant cell formation. Few actively proliferating tubules persisted between the ages 4 to 12 months, although the testes were scrotal in position. In an invigorating environment (60-68°F.) the testes were retained intra-abdominally and active germinal tissue persisted. A change to humid heat induced abrupt degeneration of germinal epithelium. Control mice displayed similar responses when exposed to humid heat. The sexual functions were quickly and profoundly affected by changes in environment, and it is believed that the general metabolic level is more important than the anatomical position of the testes. Moist heat, for example, by rendering body heat loss difficult, produced a prompt suppression of fertility, which is quickly evidenced histologically. The germinal suppression seemed associated with a general metabolic subsidence as the external humid heat made body heat loss difficult.—Hans O. Haterius.

Increasing the effectiveness of testosterone. Parkes, A. S., Lancet **231:** 674. 1936.

The effectiveness of testosterone in restoring the atrophic prostate and seminal vesicles of castrated rats can be increased by adding x-substance of pure fatty acids to the solution injected or even by augmenting the volume of oil used for injection. Thus it is found that testosterone acetate and testosterone proprionate are many more times effective than the free hormone on castrated rats. The action is so prolonged that it can be restricted to twice a week without loss of effectiveness. Of the two compounds, the proprionate has more intense and prolonged action than the acetate.—L. B. S.

The colorimetric determinations of urinary estrin. Pincus, G., Grace Wheeler, Genevieve Young and P. A. Zahl, J. Biol. Chem. **116:** 253. 1936.

A comparison was made of the colorimetric estimations of estrone, estriol and estradiol in urine with the phenol sulfonic acid test as proposed by Cohen and Marrian (A) and by Cartland et al (B). The method A gave reliable results of estriol content only during the last 3 months of pregnancy. It gave high values during early pregnancy and in non-pregnant urines for estriol and even during the last months for estrone. Method B gave cloudy solutions and the color fades too rapidly for practical use. The benzoyl chloride test of Gortz distinguishes estrone and estradiol from estriol. It gives a more intense color reaction with estrone than estradiol. The results agree with (A) for human pregnancy urines but with rabbit urines the values are twice as great. The arsenic acid test of David is specific for pure estriol but cannot be used with urine extracts.—J. M. L.

Distention, a stimulus for uterine growth in untreated ovariectomized rabbits. Reynolds, S. R. M., and S. Kaminester, Am. J. Physiol. **116:** 510. 1936.

Thirteen observations were made upon the effect of chronic uterine distention by means of paraffin pellets of known size. One week following ovariectomy, which was performed during pseudopregnancy, greased pellets were inserted aseptically into the uterus. Two weeks later appropriate segments were removed and examined histologically, together with segments which had been subjected to acute distention and non-distended segments. The authors found that chronic distention induced hypertrophy, both of the endometrium and the myometrium, the percentage increase in each being about equal. Increase in the endometrium resulted from active proliferation of the epithelial tissue; no restitution of epithelial glands was noted. The myometrium increase was due to a marked hypertrophy with probably some hyperplasia, with a decided increase in ratio of cytoplasm to nuclear material. From calculations, it was concluded that the distending influence, in order to be effective, must be somewhat greater than one-half and somewhat less than twice the size of the undistended uterus, maximal growth being obtained when the distending influence was approximately equal to the size of the undistended uterus.—Hans O. Haterius.

The hormonal treatment of acne vulgaris. Rosenthal, T., M. Clin. North America **20:** 985. 1936.

Estimation of estrogenic substance in the urine and blood in 2 independent series of females with acne yielded parallel results: 82% of patients showed absence or subnormal quantity in the urine, and 93% showed absence or subnormal quantity in the blood. The inference that associated with acne there is abnormality of formation or of utilization of the sex hormone appears justified. Treatment of these patients with estrogenic substance and gonadotropic substance produced improvement in 60% of the cases observed. It is pointed out that prolonged treatment with estrogenic substances is not without danger; sclerosis of the ovaries, and

carcinogenesis have been reported. Regardless of what specific therapy may be employed, the basic principles underlying the general management of patients with acne must not be overlooked.—I. B.

The action of progestin on the uterus of the cat. Rowlands, I. W., and M. K. McPhail, Quart. J. Exper. Physiol. **7:** 109. 1936.

Estrone administration induced development of long straight endometrial glands, with a distinct lumen and bordered by a columnar epithelium both in immature and in adult spayed females. Subsequent progestin administration caused these glands to hypertrophy and to become tortuous; the glandular mouths opened, giving the mucosa the typical fringed appearance seen in normal pseudopregnancy in the cat. Following upon hypophysectomy the quantity of progestin required to produce these responses was somewhat greater than in the spayed animal. The amount was some 70 times greater than that required in the rabbit. Considerable alveolar development occurred in mammary glands in spayed, but not in hypophysectomized cats.—Hans O. Haterius.

Hormonal factors affecting vaginal smears in castrates and after the menopause. Salmon, U. J., and R. T. Frank, Proc. Soc. Exper. Biol. & Med. **33:** 112. 1936.

In a group of 45 women, consisting of 24 surgical, 11 x-ray castrates and 10 menopausal subjects, a preliminary examination of the daily vaginal smears (5—21 days), gave consistently negative smears (abundant leucocytes and nucleated compact cells) in 18. Persistently positive smears (leucopenia and cornification) were found in 13 women (7 surgical, 2 x-ray castrates and 4 menopausal subjects). In 22 cases a study of the urine revealed a gonadotropic hormone content of 5 to 100 R.U. per day, without any relationship between the vaginal smear and the quantity of gonadotropic hormone excreted. Nor was there any correlation between symptoms and the vaginal smear. The administration of large doses of estrin (4,000 to 36,000) changed the negative to positive smears and intensified the positive character of those already positive. This effect lasted for about 4 weeks, after which there was a gradual regression to the pretreatment condition over a period of 6 to 10 weeks. Amelioration of symptoms coincided with the change in the vaginal smears, and gradual recurrence of symptoms paralleled the regression of the positive character of the smears.—M. H. F.

Connection between function of mammary gland and hormones of incretory glands (Über den Zusammenhang zwischen der Funktion der Brustdrüse und den Hormonen der inkretorischen Drüsen). Sawitzky, W., Zentralbl. f. Gynäk. **59:** 2784. 1935.

Sawitzky shows that the administration of estrogenic substance to puerperal women in most cases prevents lactation and the swelling of the breasts. If estrogenic substance is given together with the factor in pregnancy urine, this effect is even more noticeable. The author points out that the administration of these substances seems advisable when cessation of lactation is aimed at (death of the child). He thinks that this mode of treatment may eventually replace the measures formerly employed to produce cessation of lactation. His observations were made on 29 women: 15 were given only estrogenic substance and 14 were treated with both substances. Of the 15 women, 9 responded well; of the 14, 10 responded favorably. At first the author gave the injections subcutaneously but, because this procedure proved painful, intragluteal injection was resorted to. He injected 2 or 3 times daily, in all from 200 to 800 M.U. of estrogenic substance and also from 50 to 100 R.U. of the factor in pregnancy urine every day. The milk secretion ceased after 4 or 5 days. The author concedes that his observations will have to be verified on a larger material.—Abst., A.M.A. Service.

Effect of crystalline preparations of the follicular hormone on the growth of some cultivated plants (Die Wirkung von Follikelhormon-Kristallisaten auf das Wachstum einiger Kulturpflanzen). Scharrer, K., and W. Schropp, Biochem. Ztschr. **281:** 314. 1935.

Vegetation experiments in containers with various chemically and physically defined soil mixtures were carried out with wheat, rye, barley, oats, rape, beans, soya beans and sugar beets, adding increasing amounts (500 to 1,000 M.U.) of technical follicular hormone crystallisates. Phenolic and alkaline (sodium phenolate) preparations containing equillin and hippulin but no auxin were used. With summer wheat increased production of both grain and straw was obtained, sodium phenolate hormone being superior. For summer rye and summer barley not less than 1,000 M.U. of sodium phenolate hormone gave grain and straw increases. Experiments on oats were negative. Small, but definitely increased straw production of summer rape was found. Treated rape plants blossomed 3 days earlier than controls. Five hundred M.U. of phenolic hormone gave 24%, 1,000 M.U. 39% increased grain production of beans, while straw

production decreased. Sodium phenolate hormone was less active. With soya beans no well-defined production changes were obtained. The dry substance of sugar beets was increased 14% by 1,000 M.U. of sodium phenolate hormone, but sugar and leaves remained unchanged. Growth of young plants was enhanced but without correspondingly increased yields.—F. Breuer.

Further observations on the substance in the serum of pregnant women antagonistic to vasopressine (Observations complémentaires sur la substance antagoniste de la vasopressine dans le sérum de la femme enceinte).

Specificity of the substance in the serum of pregnant women inhibiting the hypertensional effect of vasopressine (Spécificité de la substance inhibant l'action hypertensive de la vasopressine dans le sérum de la femme enceinte). Schockaert, J. A., and J. Lambillon, Compt. rend. Soc. de biol. **122:** 478; 481. 1936.

Serum of pregnant women when mixed in vitro with commercial preparations of vasopressin inhibited the hypertensional effect ordinarily induced by such substances in cats. This inhibiting action was not observed when serum and vasopressin, not previously mixed in vitro, were injected simultaneously into cats. Serum of non-pregnant women sometimes had a similar though much slighter inhibiting effect on vasopressin. Pregnancy serum was found not to influence the hypertensional action of adrenalin, ephedrin, or sympathol.—E. Cutuly.

The action of extracts of prostate and of other glands on the development of eggs and chicks (Ricerche sull'azione de estratti di prostata e di altre ghiandole sullo sviluppo delle uova e del pulcino di pollo). Stefani, F., Arch. fisiol. **36:** 370. 1936.

Small doses of prostate extract produced a specific stimulation of the sexual organs, of the comb and the wattles, and caused premature puberty. This action was enhanced by simultaneous use of testicular extract. Other effects were white coloring of the plumage and increased resistance of the egg shells. The incubation period was not affected. Folliculin acted adversely to prostate extract with reference to egg shell and plumage.—A. E. Meyer.

The estrogenic activities of some synthetic phenanthrene compounds and some oxidation products of theelol. Thayer, S. A., D. W. MacCorquodale and E. A. Doisy, J. Pharmacol. & Exper. Therap. **59:** 48. 1937.

A series of 32 phenanthrene compounds were dissolved in sesame oil and administered subcutaneously to ovariectomized mice primed with theelin. Only 9-ethylphenanthrene, 1-keto-1, 2, 3, 4-tetrahydrophenanthrene and 2-phenanthreneacetic acid gave positive responses with 25 mg. doses. The phenolic dibasic acid, $C_{18}H_{22}O_5$, obtained from theelol, gave a 50% positive response at a dosage of 300 γ. The estrogenic activity of the phenanthrenes examined is relatively low as theelol assayed by the same procedure gave a 90% positive response at dosage of 1.2 γ, theelin, 100% at 0.06 γ and dihydrotheelin, 100% at 0.02 γ.—E. L.

On the specific vaso-dilatory and plain muscle stimulating substances from accessory genital glands in man and certain animals (Prostglandin and vesiglandin). von Euler, U. S., J. Physiol. **88:** 213. 1936.

'Prostglandin,' an extract of prostate, lowers blood pressure and stimulates tone and contractions of intestinal and uterine muscle strips. It is soluble in water, alcohol, acetone, ether and chloroform; dialysable; unstable at high temperatures. Losses occur on treatment with alkali, acids or free halogens. Cataphoresis experiments indicate that it has acid properties. 'Vesiglandin' from vesicular glands (also semen) of Macacus rhesus and other monkeys has many properties similar to 'prostglandin,' but its pH stability curve differs from the latter. Their possible function in regulating the expulsion of secretions from the accessory genital glands is discussed—R. L. Z.

Effects of oestrin injections on accessory reproductive organs of the male ground squirrel (Citellus tridecemlineatus). Wells, L. J., Anat. Rec. **64:** 475. 1936.

Males of this wild rodent, having a single annual cycle of breeding capacity, were injected with estrin (from human placentae) and autopsied either during the breeding season or during the period of low sexual activity. Fifteen normal males and 2 bilateral castrates were injected daily, with either 12.5 or 25 R.U. for periods ranging from 7 to 34 days. Bulbar gland, prostate gland, Cowper's glands, seminal vesicles, epididymis and ductus deferens were compared grossly and histologically with those from untreated controls. Estrin injections in normal males during the season of low sexual activity and in castrates induced gross hypertrophy

of all accessories; microscopical examinations showed this hypertrophy to be due not to a normal stimulation, such as that induced by male hormone, but to a specific 'estrin stimulation.' Epithelium of bulbar gland, collecting tubules in Cowper's gland, prostatic ducts and Cowper's ducts exhibited metaplastic stratification and cornification. Metaplastic stratification occurred in collecting tubules of the prostate. The stratified epithelium resembled cutaneous epithelium in the possession of cytoplasmic bridges. Possible evidence for transformation of connective tissue into epithelial-like cells, in response to estrin, was noted. Estrin increased the amount of smooth muscle and connective tissue in seminal vesicles, epididymis and ductus deferens. During the breeding season, estrin damaged all accessories. Specific responses to estrin at this season were less conspicuous than at other periods.—Author's Abst., Wistar Bib. Service.

The hormonal therapy of the menstrual disturbances and their theoretical basis (Die hormonale Therapie der Menstruationsstörungen und ihre theoretischen Grundlagen). Westman, A., Acta obst. et gynec. Scandinav. **15:** 233. 1935.

The anatomy and physiology of the sexual cycle and its variations in constitutional, endocrine and nervous disturbances were reviewed. Experimental results obtained in study of the effect of folliculin, corpus luteum hormone and Prolan in the ovary and hypophysis were described. In 3 cases of primary amenorrhea treated with Prolan and folliculin, there were favorable results reported in 2 cases. Of 17 cases of secondary amenorrhea reported, 3 treated with Prolan only showed no improvement; 4 received Ovex orally with improvement in 3 patients; in 5 cases, Prolan and Ovex were used with benefit in 2 instances. The best results were obtained with large doses of folliculin. Prolan therapy in 3 patients with juvenile bleeding gave definite improvement in 1 and favorable results in the other 2. Of the patients with bleeding at the climacteric, 8 were given Lutex with favorable results in 2 instances. The other 2 were treated with Prolan with favorable result in 1. The subjective symptoms of the climacteric were alleviated following treatment with Ovex.—M. R. White.

An isomer of progesteron (Über Δ_5-pregnendion-3,20), ein Isomere des Progesterons). Westphal, U., and J. Schmidt-Thomé, Ber. d. chem. Gesellsch. **69:** 889. 1936.

A modification of the method for preparing progesterone from pregnandiol, in which debromination is carried out with neutral instead of acid alcohol and zinc, leads to the formation of an isomer in which the double bond is at C_5 instead of at C_4, and is therefore not conjugated with the 3-keto group. This substance is very readily transformed into progesterone in vitro, but apparently not in vivo, since it showed no sign of corpus-luteum endocrine activity in the Clauberg test at doses of 0.6 and 2.0 mg. This discovery further emphasizes the extreme specificity of the progesterone reaction.—D. L. T.

Menstrual bleeding after corpus luteum excision, followed by estrin or progestin therapy. Wiesbader, H., E. T. Engle and P. E. Smith, Am. J. Obst. & Gynec. **32:** 1039. 1936.

During laparotomy in women, so timed as to come shortly after ovulation, the corpus luteum was extirpated, and the use of 10,000 R.U. of estrin daily for 2 to 5 days failed to delay the onset of menstrual flow. When progestin was used, 0.5 RB.U. doses did not delay the flow, but 5 U. doses for 10 days prevented flow until 2 to 4 days after cessation of therapy. This confirms in women the work already done in monkeys.—E. L. S.

The treatment of ovarian insufficiency and its results (Die Behandlung der Ovarialinsuffizienz und ihrer Folgeerscheinungen). Wimhofer, H., and G. Pissarczyk, Monatschr. f. Geburtsh. u. Gynäk. **101:** 313. 1936.

In 48 patients with ovarian insufficiency treated for 14 months with follicular hormone obtained from the urine of pregnant women, according to the method of Zondek, some were treated with the hormone alone and others with diathermy and operation. In 33 patients with dysmenorrhea, 21 received 36,000 M.U. of folliculin and 9 diathermy treatments; 6 were permanently improved, 8 temporarily and 7 showed no improvement. Among 12 operated, 6 were permanently improved, 2 temporarily and 4 showed no improvement. Among 8 patients who received folliculin plus operative treatment, 4 were permanently improved, 1 temporarily and 3 showed no improvement. Treatment for sterility and hypoplasia was inconclusive.—M. R. White.

The bill of the sparrow as an indicator for the male sex hormone. I. Sensitivity. Witschi, E., Proc. Soc. Exper. Biol. & Med. **33:** 484. 1936.

The bill of the castrate male sparrow was used as an indicator for male hormone, following the report of Koch. The injection of preparations of known potency revealed the sparrow unit to be one-tenth of the rat unit and about one-half of the capon unit (Koch). Although the bill

of the female and of the normal male, except during the breeding season, resembles the bill of the castrate male and responds well to the extracts, the castrate male sparrow is recommended for assay.—M. H. F.

Ovaries of immature female rats receiving pregnancy urine extract and combinations of pregnancy urine extract and oestrin. Wolfe, J. M., Proc. Soc. Exper. Biol. & Med. **34:** 26. 1936.

A single injection of 25 units of APL into immature rats produced little or no increase in ovarian weight, although corpora lutea were produced. The injection of 200 U. of estrin daily for several days following the dose of APL markedly increased the ovarian weight and increased the mean diameter of the corpora lutea produced without increasing the number of the latter. The daily injection of 25 U. of APL for 10 days produced considerable increase in ovarian weight (4-fold) and marked formation of corpora lutea. However, the simultaneous injection of estrin along with the APL augmented the ovarian weight increase and increased the mean diameter of the corpora lutea.—M. H. F.

The double action—masculinizing and feminizing—of androsterone on the genital tract of chick embryos (La double action—masculinisante et féminisante—de l'androstérone sur le tractus génital des embryons de poulet). Wolff, E., Compt. rend. Soc. de biol. **121:** 1474. 1936.

Of 30 chick embryos treated with androsterone 15 developed into intersexes, 10 into genetic males and 5 into genetic females. Morphological modifications induced in the genital tracts of these animals are described. The feminizing action of androsterone is said to be similar to that of estrone.—E. Cutuly.

Estrogenic action of androsterone (Sur l'action oestrogène de l'androstérone). Wolff, E., and A. Ginglinger, Compt. rend. Soc. de biol. **121:** 1476. 1936.

Three spayed adult mice were injected with a total dose of 30, 15, and 10 mg. androsterone, respectively. Vaginal estrus lasting for several days occurred in all 3 animals. Androsterone cannot, therefore, be considered as a specifically 'male hormone.'—E. Cutuly.

The effect of artificial pseudomenstruation and menstruation on the increased elimination of Prolan A in the absence of ovarian function. Zondek, B., Am. J. Obst. & Gynec. **33:** 96. 1937.

Reports on 2 patients with primary amenorrhea, 2 with secondary amenorrhea, and 1 castrate, treated with dimenformon, and in 4 cases with progestin also, indicate that flow can be produced. In 1 case, primary type, a curette specimen showed progestational changes. The excess of prolan is recorded as "more than 111 mouse units per liter." In several cases reductions to lower concentrations (amount not specified) occurred after therapy.—E. L. S.

Inhibitory effect of testosterone propionate on experimental prostatic enlargement. Zuckerman, S., Lancet, **231:** 1259. 1936.

It is known that daily injections of an adequate amount of estrone daily into immature male rhesus monkeys will regularly result within 14 days in general enlargement of the prostate, relative diminution in the number of prostatic glands, stratification of the uterus masculinus, growth of fibromuscular tissue, alterations in the epithelium of the seminal vesicles, and swelling of the scrotal sac and surrounding skin. Eleven immature male rhesus monkeys were injected once daily for periods of 12-21 days, with an oil solution containing 100 μ of crystalline estrone. They were also injected with male hormone compound. Tissues were removed the day following the last injection and compared with those of a normal series of controls. The results showed that if the total amount given is equivalent by weight to 7 or more times the amount of estrone administered, testosterone propionate will inhibit the effects which daily injections of estrone have on the prostate and urethra of immature rhesus monkeys. According to these experiments, testosterone propionate is much more potent compound than testosterone, androstanediol, or progesterone.—L. B. S.

HYPOPHYSIS

The influence upon the sexual cycle in rats of chronic administration of gonadotropic substances. Artemov, N., and A. Atabek, Bull. de Biol. et de Méd. Expér. de l'Urss. **1:** 339. 1936.

In a series of 65 rats administration of pregnancy urine was found to result in a three-phase reaction: Stimulation of sexual maturation (8-11 days); rest period; resumption of phase

1 (25-45 days after first injection). In 8 rats similar results were obtained with prolan, 5 M.U. daily. It is postulated that phase 1 is due to follicular stimulation, phase 2 to luteinization and phase 3 to acquired resistance to the urinary principles.—R. G. H.

Responses of normal and hypophysectomized rabbits to adrenaline. Bachman, C., and G. Toby, J. Physiol. **87:** 1. 1936.

Twenty hypophysectomized and a larger number of control and incompletely hypophysectomized rabbits of the same strain and breeding were studied after subcutaneous injection of 0.25 mg. of adrenalin per kg. body weight. Both fed and 48-hour fasted intact rabbits gave marked hyperglycemic responses, the excess glucose coming from both liver and muscle glycogen. The glycogen was in some cases determined both before and after the experiment. Hypophysectomy, it is suggested, interferes with the mobilization of muscle glycogen as lactic acid to the liver, and if the liver had been depleted of glycogen by only 15 hours fast there was little hyperglycemic response to adrenalin. Accumulation of peritoneal fat was as great in 11 hypophysectomized rabbits as in 6 castrated rabbits, but the hyperglycemic response to adrenalin of the latter was the same as normal, as shown by the blood sugar, liver and muscle glycogen analyses.—R. L. Z.

Anterior pituitary extracts and liver fat. Best, C. H., and J. Campbell, J. Physiol. **86:** 190. 1936.

Injection of an extract fraction, prepared from dried beef anterior pituitary, daily for 3 days was followed by a rapid increase in size and an intense fatty infiltration of the liver of fasting white rats. The average total liver fat and percentage liver fat were 892 mg. and 13.4% for treated and 244 mg. and 5.7% for controls. The increase in liver fat was accompanied by a decrease in total body fat and an increased excretion of ketone bodies. Less marked effects were obtained in fed rats. Extracts of posterior pituitary, pancreas and liver similarly prepared and injected and a commercial pituitrin had little or no effect. There were seven series of experiments with 125 test and 85 control rats. Injections of the A.P. extract increased the ketone body content of the blood of 12 normal and 13 recently thyroidectomized rabbits. In marked hypothyroidism no ketone body rise was elicited, but a response could be obtained after the animals were thyroid fed.—R. L. Z.

Alteration of the effectiveness of the ovarian hormone, and of the anterior pituitary gonadotropic hormone by changes in the acid-base equilibrium (Über die Änderung der Wirkung des Ovarialhormons und des gonadotropen Anteils des Hypophysenvorderlappens durch Störung des Säurebasengleichgewichts). Bock, K. A., Klin. Wchnschr. **14:** 1750. 1935.

Mature, castrate, white mice on a diet of oats and acidulated water of a degree sufficient to maintain a urinary pH of 5.5 to 5.9 responded to the injection of 0.8 M.U. of progynon with a full estrus smear in the majority of instances. Castrates on a diet of raw turnips and potatoes plus alkalinised water (urinary pH of 5.8 to 7.8) did not respond with a full estrus smear with 1.4 M.U. On further experiments with infantile mice it was found that 0.6 M.U. of prolan produced blood points in animals on an acid-producing regime (urinary pH. 4.8-5.6), while 1.2 M.U. were required in mice on an alkaline regime (urinary pH 6.5-7.1). Moreover, such doses of prolan were correlated with estrus smears in the acid-regime animals but not in the animals on the alkaline diet, despite the positive ovarian response. Only with higher prolan doses did the latter animals show estrus smears.—M. H. F.

The influence of pituitary extracts upon the blood fat and ketone bodies in obese individuals (Einwirkung von Hypophysenextrakten auf Blutfett und Blutketonkörper Fettsüchtiger). Borruso, B., Klin. Wchnschr. **14:** 1746. 1935.

On the morning following a carbohydrate-free evening meal, blood samples were drawn from normal and obese patients, then 100 cc. of live oil was administered per os in the fasting condition. Blood-fat and blood-ketone bodies were followed over a period of 4 hours. Later, identical studies were made on the same patients when one or another pituitary preparation was administered at the time of the oil ingestion. The obese patients showed normal fasting blood-fat levels and flatter-than-normal blood-fat curves, following the ingestion of the oil. The injection of 25 U of pituisan (a posterior lobe preparation with biologic properties identical with those of lipoitrin, an anterior lobe preparation which affects fat metabolism) depressed the blood-fat curves in the normal patients but had much less effect on the blood fat in the obese. The latter revealed normal blood-ketone levels, but the ingestion of the oil provoked a much greater rise

of blood ketones than in normal persons. The injection of the pituisan lowered the blood-ketone curves following the oil ingestion, this effect being more marked in the obese than in the normal. The injection of 2 cc. of orophysin (an anterior lobe extract) markedly increased the fasting level of blood ketones in the normal persons and had a similar, but less marked, effect in the obese. The author interprets these results as indicating a greater ability of the tissues of the obese to absorb fat and a decreased sensitivity of the diencephalic metabolic centers toward both types of pituitary preparation in the obese individuals. The greater depression of the ketone bodies in the obese by the injection of pituisan is attributed to an admixture of some insulinotropic material in the extract.—M. H. F.

The lipid metabolism of the hypophysectomized dog and the lipid and carbohydrate metabolism of the hypophysectomized-depancreatized dog. Chaikoff, I. L., G. E. Gibbs, G. F. Holtom, and F. L. Reichert, Am. J. Physiol. **116:** 543. 1936.

The purpose of the study was two-fold, namely, to compare the lipid metabolism of the hypophysectomized dog with that of the hypophysectomized-depancreatized animal and at the same time to study the carbohydrate metabolism of the doubly operated dogs. Although the lipid content of the blood obtained from some of the dogs after complete excision of the hypophysis was significantly above normal, all lipid constituents, namely, total fatty acids, phospholipid and free and esterified cholesterol, were present in normal amounts in 11 of the 16 hypophysectomized dogs studied. In the absence of all hypophyseal tissue, these various lipid constituents were contained in normal amounts in the liver. The complete excision of the hypophysis did not inhibit the rapid accumulation of large amounts of lipids in the liver following pancreatectomy (3 dogs). The removal of the hypophysis facilitated the storage of glycogen in the diabetic dog's liver (3 cases).—K. A. Brownell.

Late reactions of the pituitary gland of rabbits to superior cervical ganglionectomy (Réactions tardives de la glande pituitaire à la gangliectomie supérieure chez le lapin). Collin, R., and L. Hennequin, Compt. rend. Soc. de biol. **121:** 1405. 1936.

Data from previous studies together with those obtained from a histological study of 2 rabbit pituitaries removed 10 and 21 days after bilateral superior cervical ganglionectomy indicate that cyclical changes occur in the anterior hypophysis. At first the gland became basophilic and excreted large amounts of colloid(?), then it became chromophobic. Regeneration of the chromophobic cells occurred and the cycle was repeated. Histological details are given for the 3 lobes of the pituitary.—E. Cutuly.

Influence of lactation on the structure of the anterior hypophysis of the rat (A propos de l'influence de la lactation sur la structure du lobe antérieur de l'hypophyse du rat blanc). Desclin, L., Compt. rend. Soc. de biol. **122:** 447. 1936.

A study was made of the hypophyses of spayed lactating rats and of spayed non-lactating rats. Animals were sacrificed 15 days after their litters had been cast. The anterior pituitaries of the non-lactating group showed typical castration changes; those of lactating rats, however, were typical of pregnancy. These results suggested that physiological nervous excitation can modify the structure of the anterior hypophysis.—E. Cutuly.

Endocrine disorders, particularly of the hypophysis, in sexual psychoses (Recherches sur les troubles endocriniens et plus particulièrement hypophysaires dans les psychoses sexuelles). Drouet, P. L., P. Florentin, M. Vérain and J. Mignardot, Compt. rend. Soc. de biol. **121:** 1409. 1936.

The urine of 5 hypersexual psychotics were tested. In every case the urine gave a positive melanophore reaction in frogs; in 2 cases it gave a positive, but feeble, Aron test for thyroid-stimulating hormone; in every case prolan A and B tests yielded negative returns. Of the 5 subjects one had a normal and 4 a more or less elevated B.M.R. Interferometric tests indicated various endocrine disturbances. It was concluded that the sexual psychoses were the result of hypophyseal hyperactivity.—E. Cutuly.

The influence of the sexual hormone upon the hypophysis (L'influence des hormones sexuelles sur l'hypophyse). Eischer, A., and N. Engel, Rev. franç d'endocrinol. **14:** 203. 1936.

Injections of 75 to 2,000 M.U. of folliculin in single and divided doses in 193 rats selected for the regularity of their estrus caused the immediate appearance of estrus lasting 2 to 4 days and then a suppression of the estrus for 10 to 15 days. The ovaries lacked matur-

ing follicles. There was hyperplasia of the hypophysis with a decrease of basophilic cells. In 48 immature rats 40,000 to 300,000 M.U. did not enhance the action of luteinizing or gonad-stimulating hormones. In immature female rats 200 M.U. of folliculin retarded growth and prevented genital development. The authors conclude that folliculin affects the genital cycle of the rat by inhibiting the production of the gonad-stimulating hormone and the growth hormone of the hypophysis.—W. L. Thomas, Jr.

Clinical and experimental studies on the behavior and the significance of thyrotropic hormone in the blood (Klinische und experimentelle Untersuchungen über das Verhalten und die Bedeutung des thyreotropen Hormons im Blute). Fellinger, K., Wien. Arch. f. inn. Med. **29**, 375. 1936.

An extract was prepared from human blood which retained its thyrotropic hormone but which did not contain thyroid hormone. The hemolyzed blood was treated with 40% acetone. The thyrotropic substance was contained in the resulting filtrate. Increasing the acetone concentration to 85% gave a precipitate which contained the thyrotropic hormone. After centrifuging and washing several times with acetone the precipitate was dried in a vacuum at 40° C. The dried powder was extracted with distilled water at pH 6.0. The extract so obtained gave a powerful thyrotropic action and contained no demonstrable thyroxin. Young guinea pigs (180 to 220 gm.) were used as test animals. The total dose of extract in three equal parts was injected on consecutive days. On the 4th day the animal was killed in ether narcosis, the thyroid removed and serial sections prepared. The degree of stimulation was evaluated by histological criteria. In normal cases an amount of extract representing 5 to 8 ml. of whole blood produced a definite thyrotropic effect. Cases of hyperthyroidism showed a decided reduction of thyrotropic hormone in the blood, while hypothyroidism, when primary in the thyroid, or postoperative, showed great increase in thyrotropic hormone. Hypothyroidism accompanied by disease of the anterior hypophysis continually displayed low thyrotropic values. It is held that thyroid activity exerts an inhibitory influence on the production of thyrotropic hormone by the hypophysis.—W. J. A.

Cytoplasmic modifications in the pituitary gland of frogs kept in darkness (Modifications cytologiques de la glande pituitaire des grenouilles maintenues à l'obscurité). Florentin, P., and F. Stutinski, Compt. rend. Soc. de biol. **122**: 674. 1936.

The chromophilic lobe of the pituitary gland of frogs (Rana temporaria) normally contains acidophilic, basophilic and chromophobic cells. This lobe in tadpoles kept in darkness for a week shows a complete absence of chromophobic cells and, with the exception of a few basophiles, it is composed entirely of eosinophiles. The cells of the intermediate lobe become vacuolated and often fragment into small droplets of colloid. These observations are interpreted as indicating arrested excretion and are believed to lend support to the unitarian theory of origin of pituitary cells.—E. Cutuly.

Chromophobe adenoma of the pituitary associated with Cushing's syndrome. Fuller, C. J., Lancet **231**: 181. 1936.

A case is described presenting the following features of Cushing's syndrome: plethoric complexion; increase in subcutaneous fat in the face, neck, trunk, well-marked striae, and raised blood pressure; loss of libido and decreased sugar tolerance. An attempt was made to arrest the progress of this condition with deep x-ray therapy, but with no success. On autopsy a chromophobe adenoma of the pituitary was found, showing hyalin changes in the cytoplasm of the basophil cells of the anterior lobe.—L. B. S.

Extraction of gonadotropic factors from the blood. Improved technique. Frank, R. T., and U. J. Salmon, Proc. Soc. Exper. Biol. & Med. **34**: 363. 1936.

A method is described utilizing the precipitation of the activity from concentrated acetone. Compared with other precipitants, acetone gave the most satisfactory results. With this technique it is possible to secure a positive gonadotropic reaction from 40 cc. of blood of normal women on days 9 to 13 of the cycle.—M. H. F.

Augmentation of ovary-stimulating action of gonadotropic preparations. Hellbaum, A. A., Proc. Soc. Exper. Biol. & Med. **33**: 568. 1936.

Tannic acid preparations of normal male urine (200 cc. equivalents) markedly augmented the effect of whole sheep pituitary extract on the immature rat ovary. Augmentation was not produced when the urine extract was added to either the follicle-stimulating fraction or the

luteinising fraction derived from sheep pituitary, but was again demonstrated when these two fractions were recombined and injected with the male urine extract. The latter did not augment the effect of pregnancy urine or of pregnant mare serum. However, with whole sheep AP extract, augmentation was obtained by combination with either extracts of milk, egg white, lemon, horse thyroid or beef liver. The male urine augmentor was not destroyed by boiling but was destroyed by incineration. It was inactive by mouth.—M. H. F.

The initiation of lactation (Ueber die Entstehung der Laktation). Hoffmann, F., Zentralbl. f. Gynäk. **60:** 2882. 1936.

Fifteen women who lactated successfully showed no lactogenic hormone in ¼ the day's urine shortly before delivery, but demonstrable amounts on or after the second day of the puerperium. The authors mention that many women with unsuccessful lactation had no hormone, and some had responded to the use of Squibb lactogenic extracts of the pituitary. They question the statement that lactation is inhibited during pregnancy by estrogenic substance. They have prevented undesired lactation by doses up to 50,000 U daily, although there was some depression of breast distension at times.—E. L. S.

Hypophyseal mammary hypofunction (Ueber die hypophysäre Hypogalaktie). Hoffmann, F., Zentralbl. f. Gynäk. **61:** 35. 1937.

Continuing previous work on the urinary excretion of lactogenic hormone at term and post-partum, 8 cases of inadequate milk supply have been studied by the modified Lyons-Page technique. In 7 there was a distinctly smaller amount of the hormone than in the cases with adequate lactation.—E. L. S.

Inhibitory effect of follicular hormone on the anterior pituitary in humans. Jones, J. S., and T. N. MacGregor, Lancet **231:** 974. 1936.

An attempt was made to inhibit not only the gonadotropic but also the diabetogenic principle of the anterior pituitary by giving large doses of estrin. Ten subjects, all females, ages 52 to 83, were selected. They were all psychotic and had been in the hospital for more than 3 years. They received 1,500,000 M.U. in 60 days; 2 receiving 1,000,000 M.U. in 46 days, and 2 receiving 500,000 M.U. in 20 days. In all, the excretion of the gonadotropic hormone in the urine, which had exceeded 50 M.U. per liter was abolished. Oestrin produced bleeding in 7 out of 10 women. However, this huge dosage had no consistent effect on the glucose tolerance of the diabetogenic principle.—L. B. S.

Species—Specificity of the so-called 'anti-hormones'. Kabac, J., Bull. de Biol. et de Méd. Expér. de l'Urss. **1:** 342. 1936.

Prolan was injected into 30 female rats until the animals became non-reactive (45-60 days later) as shown by the sexual cycles. Whether the insensibility was due to anti-hormone was tested by implantation of rat hypophyses, rabbit hypophysis, gonadotropic extract of sheep pituitary, serum of pregnant mare and extract of human pituitary. A species-specificity was noted in the reactivity. Hence it is concluded that the insensitivity was due to some mechanism akin to anti-body formation.—R. G. H.

The maternal instinct after hypophysectomy (L'instinct maternel après hypophysectomie). Leblond, C. P., and W. O. Nelson, Compt. rend. Soc. de biol. **122:** 548. 1936.

Hypophysectomy of 8 pregnant mice a few days before parturition caused cessation of milk secretion, atrophy of the mammary glands and a decrease in maternal instinct. If, however, different groups of young mice were supplied to these animals twice a day their maternal instinct was renewed; in one instance it was maintained for 3 months after hypophysectomy. These results together with the observation that the presence of young animals stimulated the maternal instinct 88% of male and female mice at puberty indicated that this instinct was not dependent on the action of the lactogenic hormone of the anterior pituitary.—E. Cutuly.

The anterior hypophysis and the thyroid during ovulation (L'hypophyse antérieure et la thyroïde au cours de l'ovulation). Marza, V., and A. Blinov, Compt. rend. Soc. de biol. **121:** 1438. 1936.

During ovulation in the pigeon structural modifications occur in the endocrine glands. The anterior hypophysis undergoes hypertrophy; no increase is seen in the number of baso-

philes or chromophobes (eosinophiles were not studied by the techniques employed); colloid formation is not accelerated. The thyroid presents histological signs indicating hyperactivity, a condition not obtaining in male pigeons during spermatogenesis nor in non-ovulating female pigeons.—E. Cutuly.

The pituitary gland of Fundulus. Matthews, S. A., Anat. Rec. **65:** 357. 1936.

Sections of the pituitary gland of Fundulus showed a pars nervosa and a pars glandularis, the latter presenting a large 'transitional portion' and a narrow pars intermedia. Chromophobes, basophiles and acidophiles were found in varying numbers in the two regions of the pars glandularis. The transitional region is almost entirely chromophobic during the summer months, acidophiles appearing in the fall and persisting throughout the winter, though staining less intensely in late winter and spring. In the pars intermedia the percentage of acidophiles is highest in late May, July and September and is lowest in March and April, but considerable variation is found in individual animals. In addition deep-staining basophiles of a special sort appear in many animals, notably in those killed during summer and fall. Since little is known of the function of the teleost pituitary, correlation of the observed changes in its cellular appearance with activities of the animal must be determined by suitable experiments.—Author's Abst., Wistar Bib. Service.

The chemistry of lactogenic hormones. McShan, W. H., and H. E. French, J. Biol. Chem. **117:** 111. 1937.

The lactogenic hormone obtained from the anterior pituitary by extraction with acetic acid or acid acetone was found to be protid in nature and soluble in glacial acetic acid, formamide, 66% aqueous alkaline alcohol and partially soluble in 90% alcohol and lactic acid. The extracts were inactivated by boiling in 0.1 N. sodium carbonate 45 min., by 0.033 N. sodium hydroxide at 37° C. in 4 hours, and by 0.1 N hydrochloric acid at 100° C. in 1 hour. The material was inactivated by sodium nitrite, sodium cyanide, benzoyl chloride, and formaldehyde, but not by acetic anhydride. Peptic and tryptic digestion rendered the material completely inactive. The active protein contained about 15.0% of N_2 and 0.85% free amino N_2, 0.32 of P. and 1.5% of S. The products prepared by acetic acid and acid acetone methods of extraction are essentially identical.—J. M. L.

The site and mechanism of the antiketogenic action of insulin. Mirsky, I. A., Am. J. Physiol. **116:** 322. 1936.

A study was made of the action of 2 substances; namely, insulin and ergotamine tartrate, on the ketosis produced by the injection of a neutralized alkaline anterior hypophyseal extract. The subjects were white, male rabbits approximately 2 kg. in weight and fasted 24 hr. Sodium pentobarbital anesthesia was employed throughout all experiments. There were 6 animals in each experimental group and 6 controls. Into those in the insulin group, hypophyseal extract equivalent to 2 gm. of fresh gland and 1.3 to 3 U. of insulin were injected subcutaneously in opposite sides; those of the ergotamine group received 2.0 mg. ergotamine tartrate intramuscularly and later the hypophyseal extract. Controls received only hypophyseal extract. The results showed that both insulin and ergotamine inhibited the production of acetone bodies which normally result from the administration of neutralized alkaline extract of the anterior hypophysis. Since it has been previously proven that the hypophyseal extract is effective only in the presence of the liver, it is probable that these substances exert their antiketogenic effects on this organ. It is suggested that insulin and ergotamine owe their action to their antiglycogenolytic function.—K. A. Brownell.

An analysis of the action of posterior pituitary extracts on the uterus. Morgan, T. N., J. Pharmacol. & Exper. Therap. **59:** 211. 1937.

Optical records of the uterine movements and plethysmographic records of the total volume of the uterus of unanesthetized rabbits were obtained. Pitocin, pitressin and pituitrin in 1 U doses, were injected intravenously. Pituitrin caused an increased tonus for 4 to 5 minutes followed by a period of diminished activity. Pitocin caused an immediate contraction of the uterus and an increased tone lasting 5 to 15 minutes. Pitressin caused a transient initial contraction followed by a period of depression, lasting about 30 minutes. During this period of depression, the uterus is refractory to pitocin, adrenalin, ergotoxin and quinine but calcium chloride produced a contractile response. As pitressin causes a vascular spasm of the uterus, the author believes that pituitary preparations containing this hormone should be used in the treatment of uterine hemorrhage.—E. L.

Thyroxin sensitivity of hypophysectomized rats (Über die Thyroxinempfindlichkeit hypophysecktomierter Ratten). Reiss, M., and S. Fischer-Popper, Endokrinologie **18:** 93. 1936.

Hyposectomized rats show a definitely greater sensitivity to thyroxin than do normal animals. Under the influence of thyroxin they lose weight more rapidly and the respiratory exchange goes on much more rapidly.—W. J. A.

Maintenance of pregnancy in the hypophysectomized rabbit with progestin. Robson, J. M., J. Physiol. **86:** 415. 1936.

It has been found that hypophysectomy during the second half of the term of pregnancy in rabbits is followed by abortion within 24 to 48 hours; corpora luteal degeneration also is apparent. Sixteen mature female rabbits were hypophysectomized on the 22nd to the 24th day of exactly known pregnancy and luteal hormone injected thereafter twice daily (crude extract of Robson and Illingworth and crystalline progesterone). In 4 the foetuses remained alive and in situ for the period of normal pregnancy; in 4 the foetuses were retained, but died following operation; in 3 the administration of hormone did not prevent expulsion of uterine contents; 5 rabbits died within a few days of operation. The corpora lutea of the test animals differed only a little from normal, but the follicles were fewer and smaller than is usual after the birth of a litter. The mammary glands showed little or no development after hypophysectomy and there was no milk production in animals which carried to full term.—R. L. Z.

Neurocrine field of activity of the hypophysis (Le champ d'action de l'hypophyse par neurocrinie). Roussy, G., and M. Mosinger, Compt. rend. Soc. de biol. **122:** 643. 1936.

The distribution of hypophyseal colloid in dogs has been found to be very extensive, from which it is concluded that the hypophysis can influence by a neuro-hormonal mechanism numerous nerve centers of the thalamus and hypothalamus.—E. Cutuly.

Gonadotropic activity of the pituitaries of vitamin E deficient rats. Rowlands, I. W., and E. Singer, J. Physiol. **86:** 323. 1936.

Pituitaries from 86 E-deficient rats were desiccated, ground up and injected intravenously in aqueous suspension into 27 estrous rabbits. Assay showed the gonadotropic activity to be one-half normal (66 rats, 30 rabbits). Glands from 12 recovered E-deficient animals had normal potency when tested on 10 rabbits.—R. L. Z.

Age and the qualitative ovarian response of the immature rat to mare gonadotropic hormone. Saunders, H. F., and H. H. Cole, Proc. Soc. Exper. Biol. & Med. **33:** 504. 1936.

The injection of mare gonadotropin into 10-day-old rats produced only a growth of interstitial tissue. The same substance, at all dosage levels, produced the same result in 15-day rats. A pure follicle-stimulating response was obtained with all dosage levels (2, 16, and 500 R.U.) in 18-day-old females, not a single corpus luteum being found in 26 animals. Ovulation was obtained in 3 of 18 rats at 19 days, and, follicular growth, ovulation and corpus luteum formation were induced in the 21-day and 25-day animals. The significance of these results is tersely but adequately discussed.—M. H. F.

Means of augmenting the ovarian response to gonadotropic substances. Saunders, F. J., and H. H. Cole, Proc. Soc. Exper. Biol. & Med. **33:** 505. 1936.

The combination of zinc-sulphate with pituitary synergist (Evans) produced a 3-fold increase in ovarian weight over that obtained with the pituitary synergist alone. Significant augmentation was also obtained by the addition of casein or egg albumin to the synergist. Like the luteinising principle of Fevold and Hisaw, each of these combinations with the synergist produced heavily luteinised ovaries. It is suggested that these non-specific augmentation effects are produced by retarding the absorption of the active material.—M. H. F.

The effect of antithyrotropic serum on the thyroid gland of guinea-pigs treated with thyrotropic hormone. Scowen, E. F., and A. W. Spence, J. Physiol. **86:** 109. 1936.

The histologic appearance of the thyroid glands of guinea pigs was used as a criterion of the thyrotropic activity of anterior pituitary extract. Fifteen normal normal animals gave a constant hyperplastic response, but of 12 guinea pigs given AP extract plus normal rabbit serum 6 remained normal. The serum from 3 rabbits given 8 cc. of AP extract daily for

5 or 6 weeks inhibited the thyrotropic effect in 9 guinea pigs. Serum from a rabbit receiving AP for 3 months was slightly less antithyrotropic (3 cases). In 12 animals it appeared that normal human serum had a slight antithyrotropic effect, but serum from patients with Graves' disease had none.—R. L. Z.

The basophil cells of the anterior pituitary and their relation to high blood pressure and eclampsia (Die basophilen Zellen des Hypophysenhinterlappens und ihre Beziehungen zum Hochdruck und zur Eklampsie). Scriba, K.,—Virchows Arch. f. path. Anat. **297:** 221. 1936.

The occurrence of basophils in the posterior lobe of the hypophysis was investigated using 346 human glands. No correlation with age or with specific diseases was found, but there were fewer basophils in the posterior lobe of females than in males. The basophils are considered by the author to be infiltrated from the pars intermedia with no specific endocrine function.—R. L. Z.

The central pressor actions of pituitary hormones and of non-isotonic solutions (Über die zentrale Blutdruckwirkung der Hypophysenhormone und nicht isotonischer Lösungen). Seligsohn, A., Wien. Arch. f. inn. Med. **29:** 421. 1936.

Dogs weighing from 9 to 21 kg. were narcotized with paraldehyde or with urethane. Blood pressure was obtained from the femoral artery. By means of suboccipital puncture cerebrospinal fluid was withdrawn and other fluids introduced. It was found that pure vasopressin, when given in doses of 10 U in physiological saline, caused an elevation of blood pressure. Anterior pituitary hormones, especially the corticotropic and adrenotropic, give no appreciable rise of blood pressure, when administered by intracisternal injection. Hypotonic and strongly hypertonic solutions cause disturbances of blood pressure or of respiration. The author warns that all solutions given by the lumbar, suboccipital or intracerebral routes, whether for diagnostic or therapeutic purposes, should be carefully adjusted to be isotonic with the cerebro-spinal fluid, if reliable conclusions regarding central action are to be drawn.—W. J. A.

Pituitary growth factor—some clinical observations. Shelton, E. K., California & West. Med. **45:** 20. 1936.

An appraisal of the clinical value of pituitary growth factor is offered. This is based on several years' experience in the treatment of 1 hypophysectomized child, 4 outright dwarfs, 6 subjects of subnormal height, 1 of fugitive acromegaly, 2 of pituitary cachexia and several of obscure endocrinopathies. It appeared that dwarfism could be only partially normalized but that in cases not exceeding 50% deficiency in growth, a normal rate could be induced. In fugitive acromegaly and Simmonds' disease rapid gain in weight and strength to low normality was secured. Few untoward effects were seen. Preparations now available are worthy of use but do not justify enthusiasm.—R. G. H.

The reproductive system and its responses to ovarian hormones in hypophysectomized rhesus monkeys. Smith, P. E., H. H. Tyndale, and E. T. Engle, Proc. Soc. Exper. Biol. & Med. **34:** 245. 1936.

Hypophysectomy, during or shortly after menstruation, did not result in post-operative bleeding although the operation, performed during the middle of the cycle in the presence of a well-developed sexual skin color, was followed by gross bleeding 2 to 4 days later. Although the size of the ovaries was not greatly reduced by the operation (due to the large amount of stroma), there was marked uterine and vaginal atrophy. A 10-day treatment with 400-500 U. of estrogenic material daily caused marked development of the involuted endometrium, and of the vaginal mucosa. In 8 of 10 cases, bleeding following the cessation of the treatments, beginning 11 to 21 days after the last injection; no bleeding occurred in the remaining 2. A 10-day treatment of such animals with 2 RB.U. of progestin daily, immediately following the estrin treatment, resulted in a typical progravid endometrium. Bleeding followed on the third day after the cessation of the progestin injections.—M. H. F.

Clinical studies with the thyrotropic pituitary hormone. Starr, P., Proc. Soc. Exper. Biol. & Med. **33:** 462. 1935.

The reactions of various kinds of patients, including castrate women, partial Simmonds' disease, hypo and hyperthyroids, was variable in regard to the relation of the response to the dosage of thyrotropic hormone. However, the injection of the thyrotropic preparation did produce a rise in B.M.R. in normal and castrate women, in cases in hypothyroidism and hyper-

thyroidism, in the case of partial Simmonds' disease, and in mild myxedema. Cases of congenital myxedema failed to respond. The response in the cases of hyperthyroidism was attended by acute exacerbation of the symptoms.—M. H. F.

Transplantation of bovine hypophysis into humans (Transplante de hipófisis bovina al ser humano). Waldorp, C. P., J. R. Membrives and S. E. Luchetti, Semana méd. **43:** 1277. 1936.

The head of the animal was immediately separated from the body and submerged in formaldehyde solution. After arrival at the destination, the skull was opened under aseptic precautions and the gland placed in sterile physiological salt solution. After removal of the fibrous tissue the implantation was made into a muscle and the wound was closed immediately. No suppuration developed. The results observed exceeded considerably the expectations based on the activity contained in the implanted material. In a case of dystrophia adiposo-genitalis in an 18-year-old male a growth of 4 cm. within 1 year was obtained. The adiposity decreased and the B.M.R. increased by 16%. The genital organs developed to twice the previous size. A 26-year-old girl with infantilism and amenorrhea experienced the first menstruation 15 days after the implantation and continued menstruating regularly. Coexistent alopecia was partially relieved. A woman having amenorrhea of 3 years' duration menstruated 44 days after the second implantation. In a case of diabetes insipidus with a daily urine output of 30 to 38 liters, a temporary reduction to normal was obtained; however, it increased again to 12 liters within 15 days. Repeated implants kept the urine production at 10 liters. The B.M.R. increased from +4 to +36%.—A. E. Meyer.

Prolonged injection of a thyrotropic extract without development of refractoriness. Werner, S. C., Proc. Soc. Exper. Biol. & Med. **34:** 390. 1936.

Two thyrotropic preparations were made from beef pituitaries: 1) by a sodium sulphate method, and 2) by a modified flavianate method of Meyer. The daily injection of the sulphate preparation into guinea pigs produced an initial rise in B.M.R., accompanied by hyperplastic glandular changes, followed by a return to normal of the histological picture and the B.M.R. between the 23rd and 40th day despite continued injections. Of 69 animals injected with equivalent daily doses of the flavianate preparation, 39 of the 43 animals autopsied on the 38th day showed markedly hyperplastic thyroids with low iodin content, and had shown a steadily maintained heightened B.M.R. up to the time of autopsy. Between the 38th and the 80th day 19 of the animals were sacrificed, and of these, 14 showed continued thyroid activation, while 5 had become refractory. Treatment of the remaining 7 animals was terminated between days 80 and 88, and of these, 6 showed continued thyroid inactivation and 1 had become refractory. This marked difference in the capacities of two thyrotropic extracts to develop refractoriness despite the similarity in potency, is regarded as evidence that the development of the refractory state is not due to the exhaustion of the thyroid or to the formation of an antihormone.—M. H. F.

Antibody nature of refractoriness to injections of hypophyseal extracts containing thyrotropic hormone. Werner, S. C., Proc. Soc. Exper. Biol. & Med. **34:** 392. 1936.

In guinea pigs injected for 32 days with a sodium sulphate thyrotropic preparation of beef A.P. at a dosage level below that for thyroid stimulation, the subsequent injection of a large dose of this preparation (20 times the previous level) failed to stimulate the thyroid, thus demonstrating a refractory condition. When another series of guinea pigs was similarly treated with equivalent doses of a flavianate preparation of beef A.P., 3 of the 5 animals had not developed the refractory state as evidenced by their pronounced response to the large dose after 41 days of treatment with sub-stimulating doses. The daily injection of the flavianate preparation for 50 days into each of 2 rabbits, and for 41 days into a sheep, did not produce antibodies against the flavianate preparation when the latter was tested according to the method of Collip and co-workers. Finally, 9 of 11 ginuea pigs which had become refractory to the sodium sulphate preparation, (B.M.R. and biopsy of gland) gave a marked response to the injection of the flavianate preparation of A.P. From such data the authors conclude that the alleged antihormone formation against thyrotropic preparations is really an immune response to proteins associated with the hormone.—M. H. F.

Concerning the ovarian changes in the rabbit after hypophysectomy (Über Ovarialveränderungen beim Kaninchen nach Hypophysektomie). Westman, A., and Dora Jacobsohn, Acta obst. et gynec. Scandinav. **16:** 483. 1936.

Ovarian changes in the rabbit after hypophysectomy were compared in different groups.

(Group 1), 27 non-mated rabbits were examined from 3 to 206 days after hypophysectomy. Characteristic, atrophic changes of follicles and interstitial cells were associated with relative increase in connective tissue and atrophy of the uterus. (Group 2), 15 rabbits hypophysectomized, 25 to 50 minutes after coitus, were examined 6 hours to 6 days after operation. No follicular rupture or corpus luteum formation occurred, mature follicles became atretic 12 to 24 hours after operation. (Group 3), 16 rabbits hypophysectomized, 50 minutes to 24 hours after coitus, examined 2 to 13 days later; at 55 minutes corpus luteum formed lasted 45 days; at 60 to 65 minutes, sometimes the corpus luteum and sometimes a hemorrhagic follicle appeared. (Group 4), 26 rabbits hypophysectomized 2 to 10 days after coitus, were examined 2 to 18 days after operation. When operation was 1 day or more after coitus, the corpus luteum formed but degenerated after 45 days. (Group 5), 11 rabbits, bled varying amounts and infused with varying amounts of salt solution from 25 to 95 minutes after coitus, demonstrated the highest secretion of luteinizing hormone at 50 to 65 minutes after coitus.—J. P. P.

PANCREAS

Hypoglycemia produced by insulin in myxedematous individuals (Sur l'épreuve d'hypoglycémie insulinique chez les myxoedémateux). Baudouin, A., E. Azérad and J. Lewin, Compt. rend. Soc. de biol. **122:** 170. 1936.

The injection of insulin into normal human subjects caused a maximum decrease in blood sugar in ½ hour, after which the blood sugar gradually increased until at the end of 3 hours it returned to or exceeded pre-injection levels. In myxedematous individuals (6) insulin produced hypoglycemia which persisted for several hours. This anomalous response did not occur in 2 hypothyroid subjects to whom thyroid substance had been administered prior to treatment with insulin.—E. Cutuly.

Excessive smoking as a possible precipitating factor in diabetic gangrene. Blotner, H., Ann. Int. Med. **9:** 987. 1936.

A case report of a young male Jew (age 34) is presented. Careful oscillometric measurements and skin temperature determinations were made during a period of smoking and during a control period. Tobacco smoking produced a gradual decrease in the temperature at various points during the period of observation. Extension of the gangrene resulted in death and an autopsy confirmed the anatomical lesion.—G. W. Thorn.

Insulin resistance and roentgen irradiations of hypophyseal region (Insulinoresistenza e irradiazioni röntgen della regione ipofisario). Cannavo, L., Policlinico (sez. prat.) **43:** 1099. 1936.

Cannavo reports 2 cases of diabetes mellitus with resistance to large doses of insulin. A series of 6 roentgen irradiations of the hypophyseal region in the second case resulted in controlling the resistance to insulin. The evolution of diabetes in this case proved to be the same after the roentgen treatment as before, but the resistance to insulin having been controlled by the irradiations made the utilization of insulin by the organism possible, which eventually resulted in the patient's recovery.—Abst., A.M.A. Service.

Action of insulin on the ovarian cycle (Contribution à l'étude de l'action de l'insuline sur le cycle ovarien). Crainiceanu, A., and L. Copelman, Compt. rend. Soc. de biol. **121:** 1303. 1936.

Four groups of mature rats, each containing 5 animals in diestrus, were injected daily for 3 days with 5, 10, 15 and 20 U of insulin, respectively. Vaginal estrus occurred in all of the rats 24 hours after the first injection and continued for the duration of the injections. Four rats were injected with 1-4 U of insulin daily for 5 days. Those treated with 1, 2, or 3 U showed vaginal estrus for 10 days after the first injection with diestrus on the 11th day. Vaginal estrus in the rat treated with 4 U of insulin continued uninterrupted for 22 days. The ovaries showed intense luteinization and well-vascularized follicles.—E. Cutuly.

Observations on a substance in pancreas (a fat metabolizing hormone) which permits survival and prevents liver changes in depancreatized dogs. Dragstedt, L. R., J. Van Prohaska, and H. P. Harms, Am. J. Physiol. **117:** 175. 1936.

A fat-free alcohol extract of fresh beef pancreas was prepared. One and one-half gm. of this extract (the amount obtained from 100 gm. of fresh gland) was shown to be effective in preventing or relieving fatty infiltration and degeneration of the liver in depancreatized dogs

(14 animals tested). The authors believe this to be a new hormone which is concerned with normal transport and utilization of fat. The name 'lipocaic' is suggested.—F. A. Hitchcock.

Experiences with liberal carbohydrate diets in diabetic children. Dwyer, H. L., South. M. J. **30:** 74. 1937.

The records of 46 children, 21 of whom had received both low and high carbohydrate diets, were studied. The high carbohydrate diet closely approximated the normal diet. The children on it showed a more stable blood sugar level, less tendency to complications, and were more careful and cooperative about following their diet. In some cases less insulin was required than when less carbohydrate proportionally was given. The diet consisted of 3 gm. of carbohydrate and 1 gm. each of fat and protein for each pound of body weight.—J. C. D.

The effect of zinc and aluminum on the hypoglycemic action of insulin. Fazekas, J. F., and H. E. Himwich, J. Pharmacol. & Exper. Therap. **58:** 260. 1936.

Zinc, 13 mg. as zinc sulfate, mixed with 10 U. of insulin and injected subcutaneously into 5 unanesthetized dogs, abolished the lowering of the blood sugar. If this amount of zinc was injected separately in a different subcutaneous area at the time of injection of insulin no inhibitory action on the hypoglycemia was produced. Aluminum had a similar anti-insulin action. In 10 cats, anesthetized with nembutal, receiving 2 U. of insulin per kg., hypoglycemia was produced. Calcium chloride and magnesium sulfate did not prevent the hypoglycemic response to insulin.—E. L.

The relation of pancreatic juice to pancreatic diabetes. Harms, H. P., J. Van Prohaska, and L. R. Dragstedt, Am. J. Physiol. **117:** 160. 1936.

The complete withdrawal of pancreatic juice from 6 dogs by means of total external pancreatic fistulae caused no hyperglycemia or glycosuria. The oral administration of fresh dog pancreatic juice (300 to 1100 cc. per day) to completely depancreatized dogs (6 animals) failed to lessen the severity of the diabetes.—F. A. Hitchcock.

Studies in diabetes mellitus. V. Heredity. Joslin, E. P., L. I. Dublin, and H. H. Marks, Am. J. M. Sc. **193:** 8. 1937.

An analysis of the case material of 6357 diabetics seen in Dr. Joslin's clinic during the years 1897-1928 is presented. It appeared that the percentages of diabetic patients with a positive family history of diabetes are far higher than those for non-diabetic control groups. Thus, taking parents between ages 30 and 59, 8.6% of the parents of diabetics were also diabetic, compared to 0.5% of those of non-diabetics; and of the parents over 60, 9.3% of the parents of diabetics were also diabetic, but only 3.8% of the parents of non-diabetics. Similar differences were found for siblings of diabetics and non-diabetics. The incidence of diabetes in the parents and grandparents of diabetic children was found to be from approximately 2 to 2½ times the normal, according to the basis used in the estimate. The predisposition to diabetes seems to be inherited as a Mendelian recessive character.—R. G. H.

The treatment of diabetes mellitus with protamin insulinate. Moller, E., and Agnete M. Thomsen, Acta. med. Scandinav. **89:** 308. 1936.

A comparative study was made of the effect of regular insulin and protamin insulinate in 10 diabetic patients. It was found that protamin insulinate allowed a saving of approximately 28% in the amount of insulin, reduced the fluctuation in blood sugar, and decreased the number and severity of the hypoglycemic attacks which, however, were more protracted when they did occur.—J. M. L.

Influence of insulin on the motor chronaxie of the rabbit (Über den Einfluss des Insulins auf die motorische Chronaxie des Kaninchens). Reiners, H., Klin. Wchnschr. **15:** 199. 1936.

The chronaxie of the flexor group of the forelimb of rabbits was determined before and after the injection of 1.5 U. of insulin per 100 gm. body weight. In some experiments there was a decrease in chronaxie during the first hour after the injection, but in all there was an increased chronaxie above the normal levels which appeared 2 or more hours after the injection. The rheobase was at first unaltered, then decreased, there being no correlation between the change in chronaxie and change in rheobase. The secondary change in the chronaxie (rise above normal) was diminished by ergotamine. In view of this, the secondary change in chronaxie is associated with the adrenin response to the insulin injection.—M. H. F.

Arteriosclerosis and hypertension in diabetes. Root, H. F., and T. P. Sharkey, Ann. Int. Med. **9:** 873. 1936.

This report is based upon an analysis of the clinical records and postmortem examinations of 175 diabetic patients at the New England Deaconess Hospital. A series of 170 non-diabetic autopsies examined by the same pathologist served as the basis of comparison. A striking excess of advanced arteriosclerotic lesions occurred in the diabetics. As an example, sclerosis with occlusion occurred in 40% of the diabetics and in only 13% of the non-diabetics. Hypertension occurred more frequently among the diabetics and in the hypertensive cases it appeared that the diabetes preceded the hypertension in most instances. Chemical examination of the aortae revealed that the cholesterol content in diabetic cases was 8.07 gm. % as compared to 4.8 in the non-diabetic. The calcium content was 5.2 gm. % in the diabetic as compared to 1.57 in the non-diabetic. Retinal sclerosis was found to occur no more frequently among the diabetics than among non-diabetic patients. The striking difference, however, was the frequent occurrence of retinal hemorrhage associated with diabetes. Pancreatic arteriosclerosis occurred no more frequently in diabetic than in non-diabetic patients. The authors present an extended discussion regarding the possible factors influencing the occurrence of hypertension, arteriosclerosis, and vascular lesions in the diabetic patient.—G. W. Thorn.

Preparation of vagotonine free of insulin (Préparation de vagotonine parfaitment débarrassée d'insuline). Santenoise, D., T. Brieu and E. Stankoff, Compt. rend. Soc. de biol. **121:** 1420. 1936.

Vagotonine is repeatedly precipitated from pancreatic extracts by LiCl and further purified by treating with potassium ferrocyanide.—E. Cutuly.

Studies on the constitution of insulin. Properties of reduced insulin preparations. Stern, K. G., and A. White, J. Biol. Chem. **117:** 95. 1937.

Insulin was treated with thioglycolic acid for varying periods of time. The number of liberated SH groups was determined by the use of phosphotungstic acid, and the viscosity was measured. The amount of cysteine liberated was directly proportional to the time elapsed. During reduction there was a marked increase in viscosity so that gel formation occurred after 7 to 12 hours at 30° C. The insulin retained about 50% of the physiological activity after treatment for 3 hours. It was suggested that some of the dithiolinkages have a special function and are not active groups but activating groups.—J. M. L.

PARATHYROIDS

Hyperparathyroidism in kidney disease. Highman, W. J., Jr., and B. Hamilton, J. Clin. Investigation **16:** 103. 1937.

Evidence was found to indicate excessive parathyroid hormone content in the blood of 20 of 23 subjects having chronic renal disease, while 38 normal controls showed no values above their normal limits. No correlations could be established between the results and the elevation of blood urea or inorganic phosphorus. The authors believe the rise in serum phosphate is the initiating factor.—E. L. S.

Hyperparathyroidism complicated by hyperthryroidism. Report of a case. Noble, J. F., and J. F. Borg, Arch. Int. Med. **58:** 846. 1936.

A case of hyperparathyroidism characterized by rapid decalcification and recalcification of bone is reported in an unmarried woman of 33. The patient has remained in good health for 2 years after removal of a parathyroid adenoma. The picture was complicated by hyperthyroidism the presence of which is believed to have influenced the course of the disease.—I. B.

The action of acid and alkali on parathyroid hormone. Tweedy, W. R., G. H. Smullen and W. P. Bell, J. Biol. Chem. **116:** 163. 1936.

Parathyroid hormone was hydrolyzed by boiling in 0.05N HCl, or in 0.03N NaOH. There was a close parallelism between the increase in free-amino groups and the loss in potency of the hormone during acid hydrolysis. NaOH caused a liberation of NH_3 and a 67 per cent decrease in potency. The results indicate the protein nature of the hormone.—J. M. L.